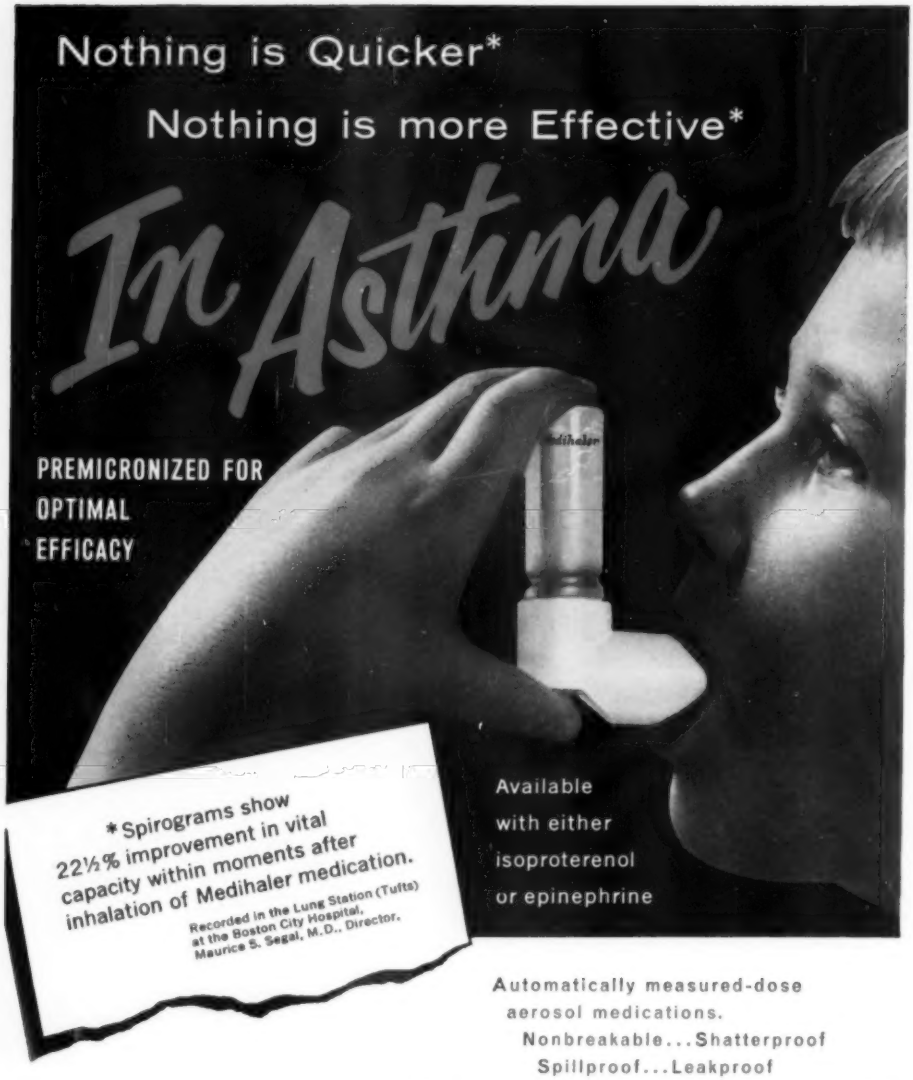
VOLUME 98 • NOVEMBER 1959 • NUMBER 5

A.M.A. JOURNAL OF
DISEASES OF CHILDREN

Society for Pediatric Research

# Twenty-Ninth Annual Meeting
# Buck Hill Falls, Pa.
# May 8-9, 1959

AMERICAN MEDICAL ASSOCIATION PUBLICATION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# A. M. A.
# Journal of Diseases of Children

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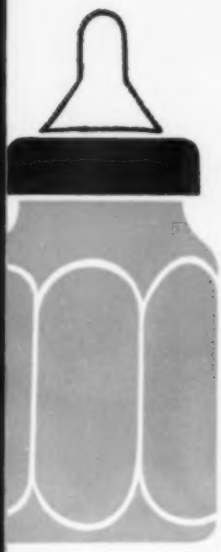

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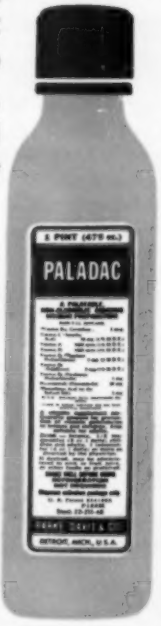


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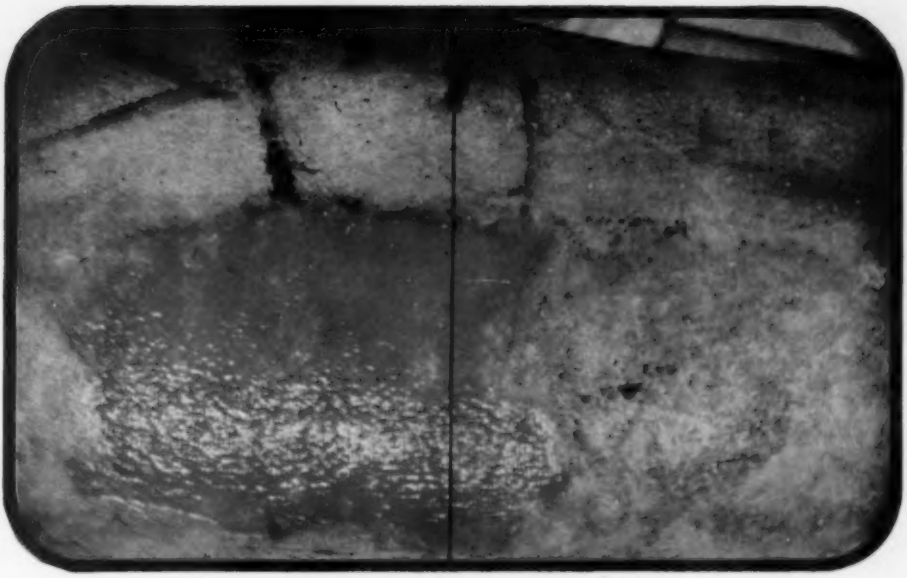

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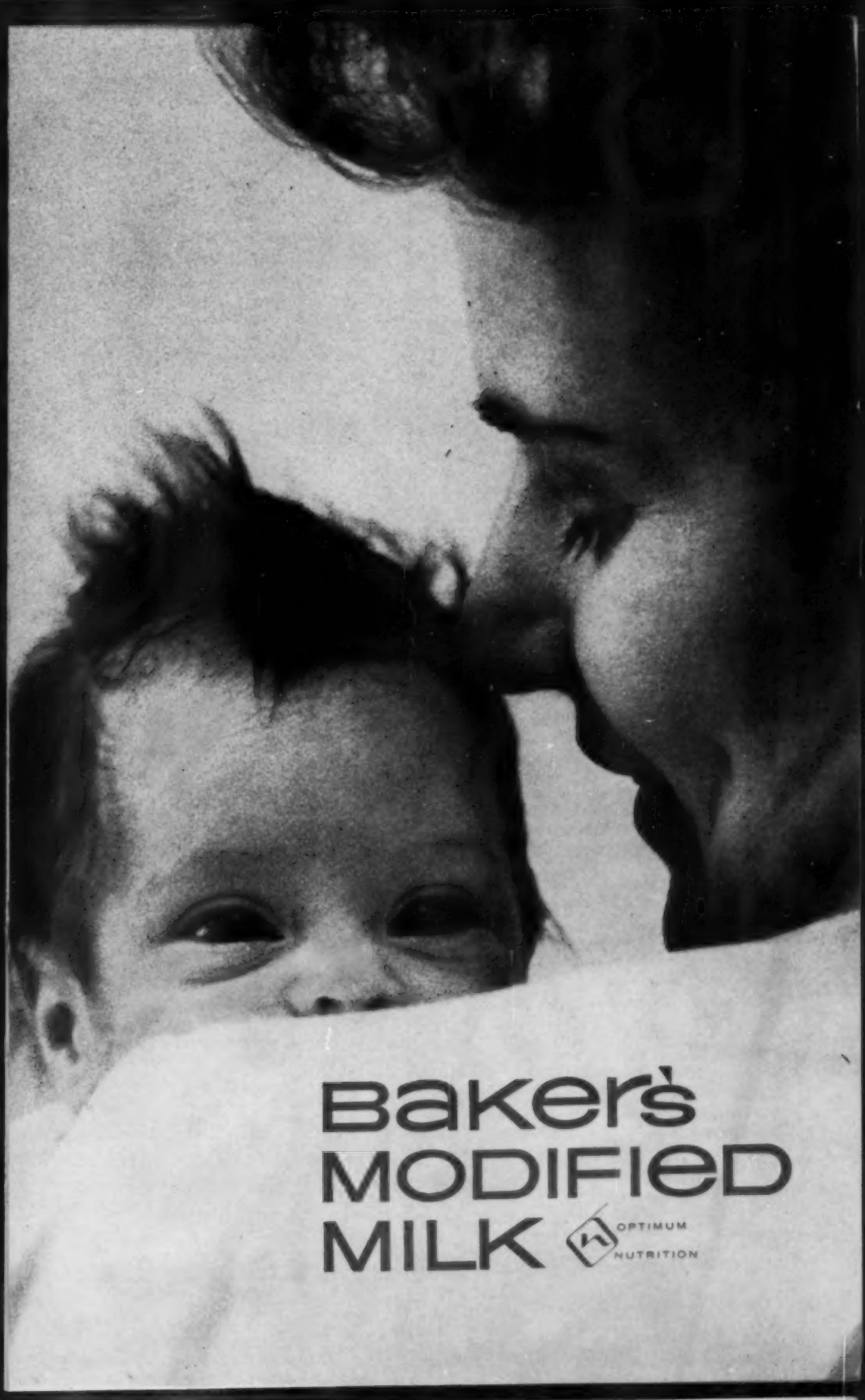
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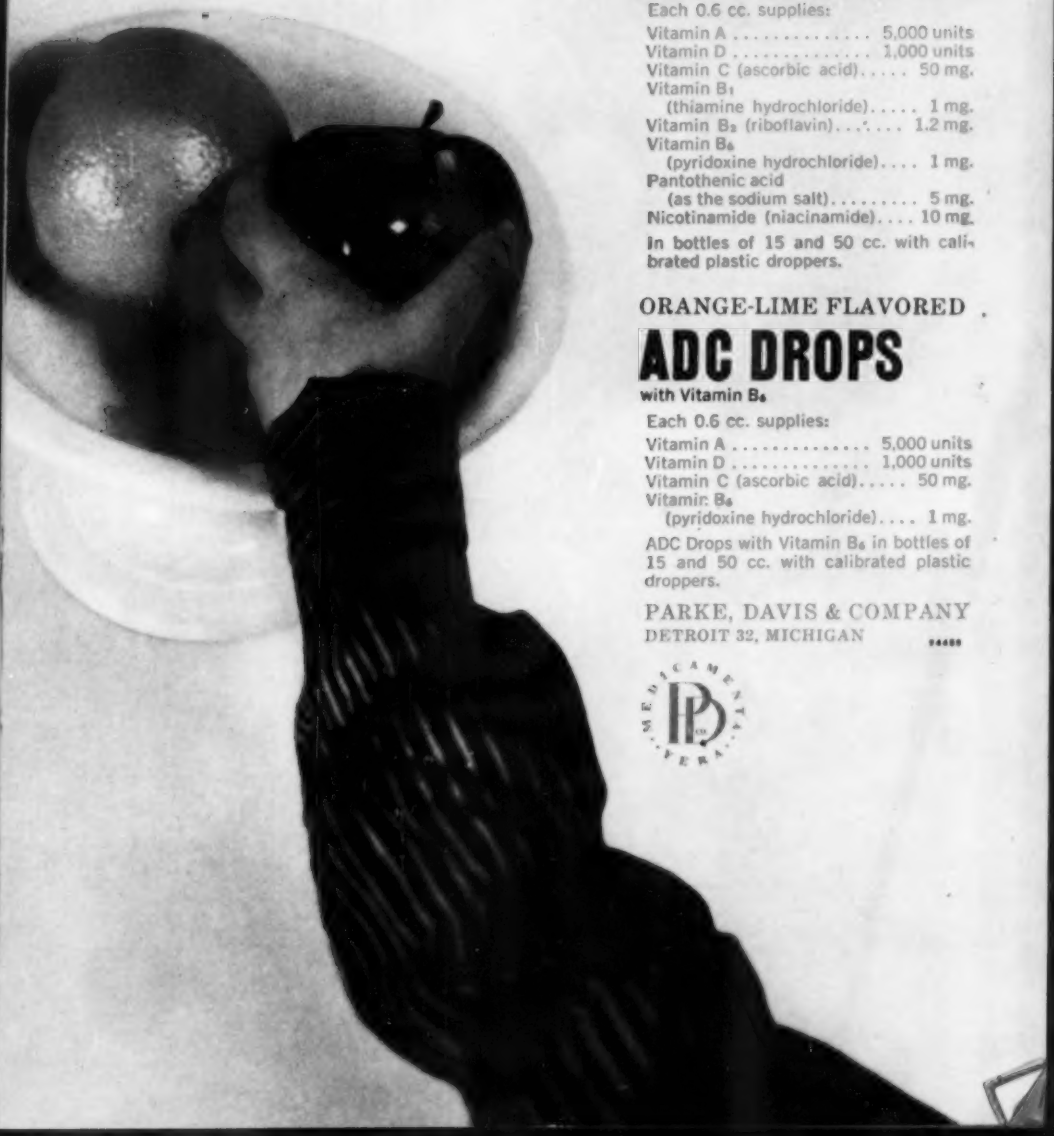

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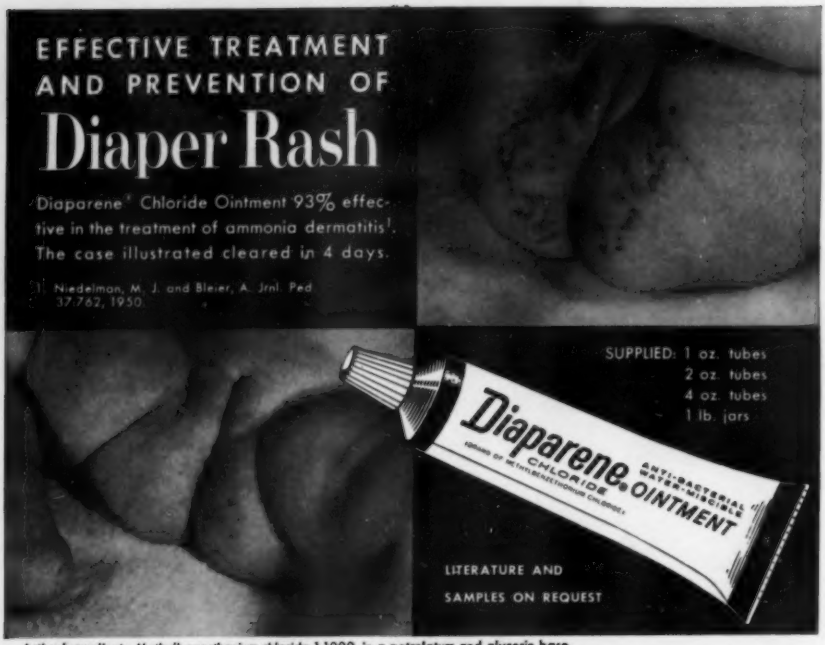


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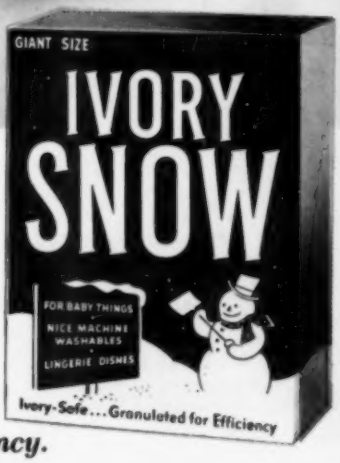


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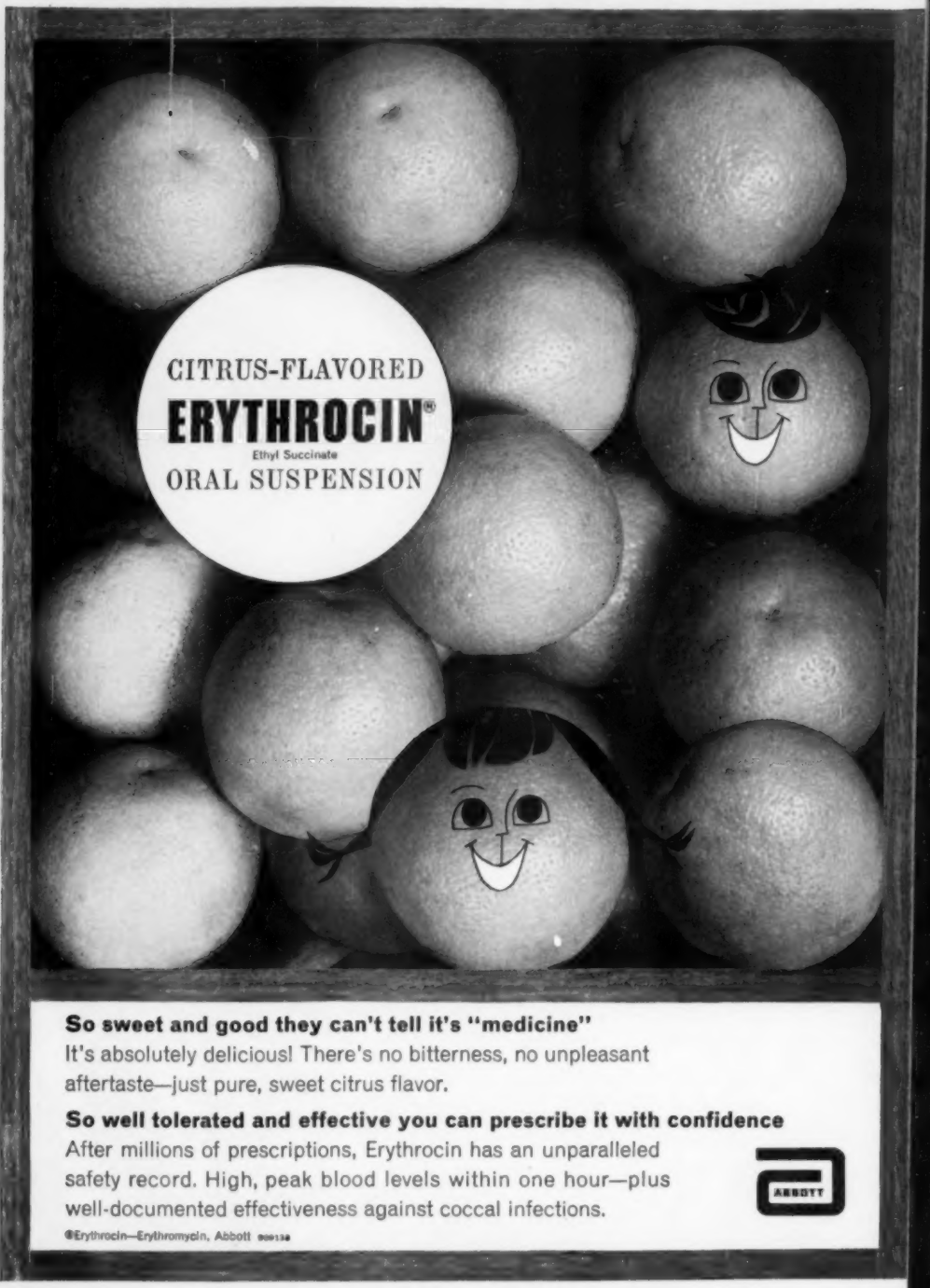


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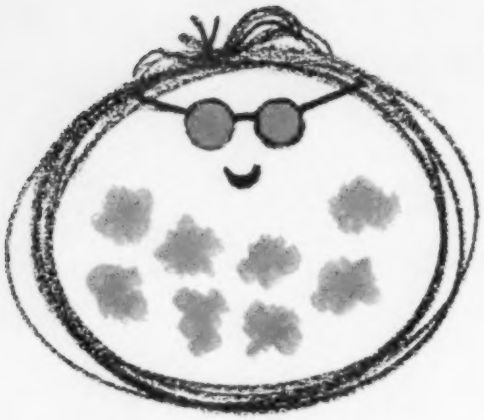
*FRIES, J. H., MILK ALLERGY – DIAGNOSTIC ASPECTS AND THE ROLE OF MILK SUBSTITUTES, J.A.M.A., NOV. 23, 1957.



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A.M.A JOURNAL OF
DISEASES OF CHILDREN

# The Society for Pediatric Research

SYDNEY S. GELLIS, M.D., Boston

If you will look back over the presidential addresses of this Society, you will note that by and large they have consisted of reviews of the speaker's own investigative work. Of late years there has been an emergence of philosophical papers in which the speaker has dwelt on certain aspects of research, teaching, and so forth. In casting about for a presidential address for the present meeting, I quickly became convinced that I would bore no one with a discussion of various aspects of liver disease or infants of diabetic mothers. Instead, it seemed appropriate to talk about ourselves, in view of the fact that 1959 marks the 30th birthday of the Society for Pediatric Research, its first meeting having been held on March 23, 1929. (This is our 29th meeting, one meeting having been omitted during the war.) Thus, this 30th birthday seems the proper time to review the conception of the Society, its birth, its neonatal course, and its development to its present manhood. I say manhood guardedly; not because I question that the Society has reached man's estate, but because I am uncertain as to the sex which is conferred on societies—are they male, female, or intersex?

Received for publication July 20, 1959.

Presidential address delivered before the Society for Pediatric Research at the 29th Annual Meeting, Buck Hill Falls, Pa., May 8, 1959.

Professor and Chairman of the Department of Pediatrics, Boston University School of Medicine; Director of Pediatrics, Boston City Hospital.

From the scanty and very dusty available early records it appears clear that the concept of the Society developed following a conversation between Dr. Kenneth Blackfan and Dr. Charles McKhann on the lack of opportunity for young men to present their work, as a result of which the latter was encouraged to visit the eastern centers, including Boston, New Haven, New York, Philadelphia, and Baltimore, to discuss with potential members the formation of a new society. At the first meeting on March 23, 1929, which was held in Boston, the following officers were elected: Dr. James L. Gamble of Harvard, President; Dr. Bengt Hamilton of Johns Hopkins, Vice-President; and Dr. Charles F. McKhann of Harvard, Secretary-Treasurer. The accompanying photographs (Figs. 1 and 2) reveal the original mem-

Fig. 1.—Top row: Darrow, Levine, Weymuller, Butler; Middle row: Gamble, McQuarrie, Drake, McKhann; Bottom row: Bridge, Josephs, Wilson, Wilder.

Fig. 2.—Top row: Trask, Holt, Guest, Stokes, Johnston; Middle row: Hoag, Stuart, Hamilton, Lawson; Bottom row: McIntosh, Byers, Anderson, Higgins.

bers present at this first meeting, the photographs having been taken in the Ether Dome of the Massachusetts General Hospital. Why they were separated into two groups I do not know nor have I been able to find out. (I was subsequently informed that the camera belonged to Dr. Harold Higgins and was unable to include all members in one sitting.)

The organization was formed primarily as an interurban group for younger men who were dependably launched in the field of pediatric research and who, because of their youth, did not at that time have access to the American Pediatric Society, the American Society for Clinical Investigation, or the Association of American Physicians. Many of the present membership are probably unaware of the fact that the Society originally began as the Eastern Society for Pediatric Research, as shown in the first printed program, that of the 1930 meeting (Figs. 3, 4, 5, and 6).

After the 1931 or third meeting, which was held in Baltimore, the problem arose of electing to membership a well-qualified physician who had one flaw—he lived west of the Charles. An eloquent plea was made on his behalf; as a result, a motion was offered to make the Society a national organization, and this motion was carried by a single vote. It is of interest that the newly elected member indirectly responsible

THE

*Eastern Society*

FOR

*Pediatric Research*

SECOND ANNUAL MEETING

BALTIMORE

MAY 3, 1930

Figure 3

**MORNING SESSION**

**9:30 a.m. — 12:30 p.m.**

Changes at the ends of long bones in lead poisoning.

L. KAJDI, D. JACKSON, and E. A. PARK

The biological relationship between human, goat and cow casein.

A. F. ANDERSON, O. M. SCHLOSS, and H. STUART

Distribution of phosphorous compounds in the blood, and variations in that distribution in certain pathological conditions ..... G. M. GUEST

The solubility of the gonococcus in alkaline media and its possible therapeutic use ..... W. L. BRADFORD

Introduced by I. McQUARRIE

Studies on pneumonia in children:

I. Mortality and blood cultures in pneumococcus pneumonia.

C. O'DONOVAN, JR., and D. M. MOORE

Introduced by J. D. TRASK

**INTERMISSION**

A preliminary discussion regarding the effect of lack of saliva on life and growth of the child and the young pig ..... T. S. WILDER and J. STOKES, JR.

Metabolic studies in an infant with absence of the ascinar tissue of the pancreas ..... D. C. DARROW

The carbon-nitrogen ratio in urine in relation to differing levels of protein intake ..... A. S. ROSS

Introduced by J. L. GAMBLE

Observations on calcification in vitro.

L. E. HOLT, E. G. NICHOLS and D. JACKSON

Continuous temperature records of infants and children under experimental conditions.

J. STOKES, JR., A. MACBRYDE and C. TATNALL

Figure 4

for the change from an interurban club to a national society was never apparently aware of his significance, never attended a meeting, and never paid his dues. At the 1932 or fourth meeting the program carried for the first time the new name of the group —the Society for Pediatric Research.

The original constitution emphasized that the Society was established for young men. It read as follows: "The name of this organization shall be the Eastern Society for Pediatric Research." Article II read: "The purpose shall be to foster pediatric investigation and to provide opportunity for younger men to present their work." Article III read: "Membership shall be of two types: active and honorary. Active members shall be limited to those individuals actively engaged in clinical or laboratory investigations in pediatrics. Honorary members may be individuals transferred from the active list at the discretion of the

## AFTERNOON SESSION

**2 p.m. — 5 p.m.**

The relation of basal metabolism to basal insensible perspiration in infants ..... S. Z. LEVINE

Some further observations on vomiting states ..... J. A. JOHNSTON

A combined study of the water balance and the mineral balance in epileptic children.
I. MCQUARRIE, C. HUSTED, and R. C. MANCHESTER

The mechanism of the ketogenic diet in epilepsy ..... E. M. BRIDGE

Total acid-base balance of a normal child on diets which increase in fat content ..... C. A. WEYMULLER and S. RATNER

## INTERMISSION

The effect of state of hydration upon the insensible water loss.
R. C. MANCHESTER, C. HUSTED, and I. MCQUARRIE

Plasma volume and electrolyte changes in dehydration ..... R. MCINTOSH

The effect of sodium chloride injections on the mineral metabolism of normal infants ..... B. HAMILTON

Intestinal Excretion ..... H. L. HIGGINS

Fractionation of plasma proteins ..... A. M. BUTLER

## EVENING SESSION

*(for members only)*

**7 p.m.** Dinner.

**8 p.m.** Business meeting.

Figure 5

executive committee or may be active members who have retired voluntarily." You will note that although the purpose of the Society was to foster pediatric investigation and to provide opportunity for younger men to present their work, the constitution made no mention of an age limit for active membership. Not until 1932, when the Society dropped "Eastern" from its name and considered itself a national society, did the membership think to protect itself from the older men, and in the 1932 revision of the constitution we read for the first time in Article III, Section 2: "Emeritus members may be individuals transferred from the active list at the discretion of the Executive Committee or may be active members who have retired voluntarily. Members attaining the age of 45 shall automatically become honorary members." Since the start of the Society in 1929 there has been no change in the professed purpose of the Society and

**EASTERN SOCIETY FOR PEDIATRIC RESEARCH**

*Honorary Member*

K. D. BLACKFAN

*ACTIVE MEMBERS*

BALTIMORE—E. A. Park, P. G. Shipley, B. Hamilton, R. McIntosh, L. E. Holt, H. Josephs, F. Bridge.

BOSTON—J. L. Gamble, H. L. Higgins, B. Crothers, A. M. Butler, R. K. Byers, F. B. Talbot, C. F. McKhann, G. M. Lawsen, H. C. Stuart.

NEW HAVEN—G. F. Powers, J. D. Trask, D. C. Darrow.

NEW YORK—O. M. Schloss, A. F. Anderson, L. A. Hoag, S. Z. Levine, C. Weymuller.

PHILADELPHIA—J. W. Stokes, Jr., T. S. Wilder, J. McK. Mitchell.

ROCHESTER—S. W. Clausen, I. McQuarrie.

TORONTO—F. F. Tisdall, T. G. H. Drake.

MONTREAL—S. G. Ross.

CINCINNATI—G. M. Guest.

DETROIT—J. A. Johnston.

SYRACUSE—J. R. Wilson.

Breakfast—The Little Inn—506 N. Broadway.

Luncheon—1 p.m.—Henry Phipps Psychiatric Clinic.

Dinner—7 p.m.—16 West Hamilton Street.

Figure 6

since 1932 the dividing line between youth and old age has been faithfully maintained at 45 despite the ill-concealed anguish of those who feel that constipation and senility do not necessarily go hand in hand.

There is insufficient time to linger on the early meetings of the Society. However, just as a presidential address without slides is an empty affair, so is an address without data. As I reviewed the initial programs of the Society I became interested in the concentration of research in the field of metabolic study. I was also intrigued with a letter dated Jan. 19, 1928, written by Dr. Daniel C. Darrow in the days when the Society was being formed, and he was discussing potential members. I quote, entirely without permission: "I would only suggest that there are too many metabolites in proportion to the bacteria. I would suggest that Trask will feel rather lonely, being the only bacteriologist. Of course the trouble is that there are not but two or three bacteriologists working in pediatrics, in spite of its importance."

In Table 1, I have attempted to show what has happened to the metabolites. If the percentages do not always total to 100%, it is because occasional papers defied categorization. You will note that, whereas metabolic problems were the subject of 63% of the papers submitted in the five-year period from 1929 to 1933, the percentage has steadily fallen until in the 1954-1958 period it constituted only 31%. Bacteriology quickly rose to a respectable

TABLE 1.—*Categories of Papers Submitted to Society for Pediatric Research; Percentage per Five-Year Period*

| 5-Year Periods | 1929-1933, % | 1934-1938, % | 1939-1943, % | 1944-1948, % | 1949-1953, % | 1954-1958, % |
|---|---|---|---|---|---|---|
| Electrolytes + Metabolism | 63 | 48 | 41 | 36 | 31 | 31 |
| Bacteriology + Immunity | 14 | 22 | 19 | 21 | 15 | 9 |
| Virology | 2 | 4 | 5 | 12 | 11 | 8 |
| Hematology | 6 | 10 | 7 | 7 | 12 | 13 |
| Neurology | 6 | 1 | 0 | 5 | 4 | 4 |
| Respiratory Physiology | 2 | 2 | 1 | 1 | 4 | 6 |
| Endocrinology | 3 | 2 | 10 | 2 | 9 | 12 |
| Cardiology | 0 | 6 | 9 | 8 | 5 | 8 |
| Gastroenterology | 2 | 1 | 5 | 2 | 4 | 5 |
| Psychiatry | 0 | 0 | <1 | <1 | <1 | <1 |

level but during the past five years appears to be on the downgrade. In all likelihood it will reach a new high in the next five years. Virology appears to have passed its peak as far as interest on the part of our members is concerned. Its stock should increase. Hematology has been a rather steady blue chip through the years, as has cardiology. It is obvious from examining the record that respiratory physiology is on its way up and that neurology, gastroenterology, and psychiatry require more attention from the membership. These appear to be—if I am to continue the stock-market lingo into which I have slipped—areas of good growth potential, worthy of investment.

It is obvious that the Society has grown tremendously. A photograph of the present membership would require almost an aerial camera. This growth has grieved many of the older members, who view with nostalgia the good old days when this was an inter-urban club. Such members, cast in the role

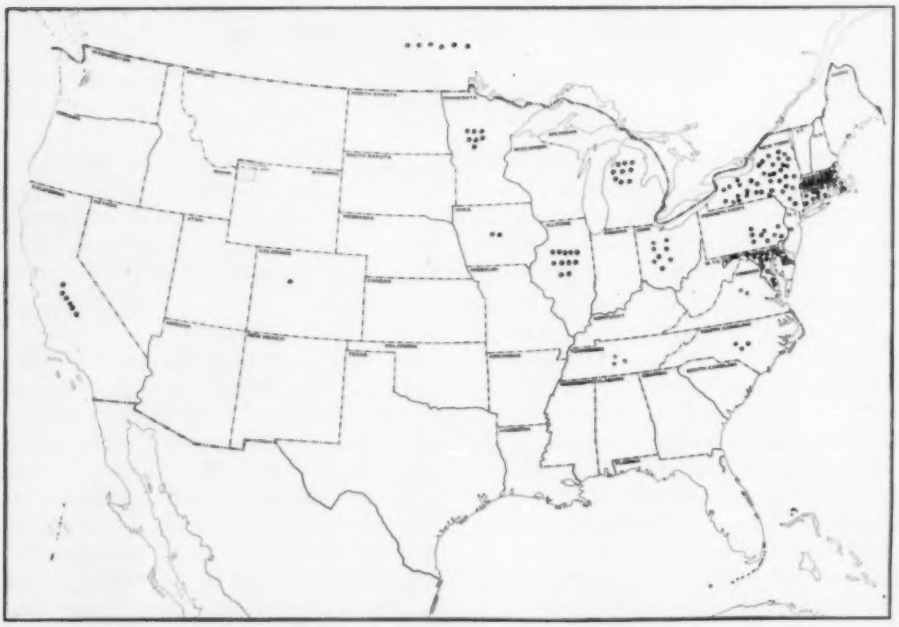

Fig. 7.—Distribution of membership in 1944.

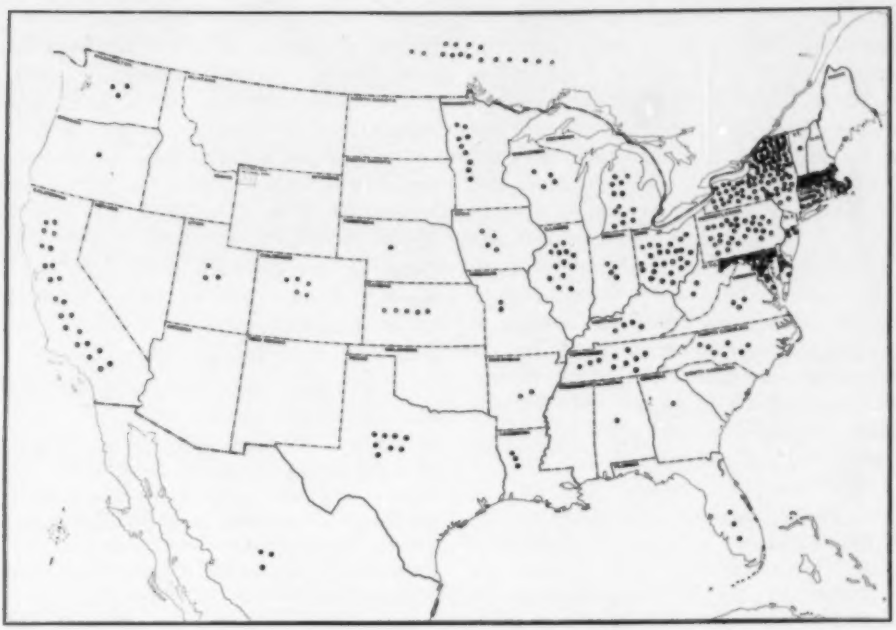

Fig. 8.—Distribution of membership in 1959.

of Adam, would probably think back to the good old days when man did not exist. Figure 7 shows the distribution of the membership in 1944 when we were 15 years old and can be compared with Figure 8, which shows our present distribution. At our 15th birthday our membership still gave the appearance of an eastern society. Today we are to be found in Mexico, Canada, and all states except those which are not yet "blessed" with medical schools. Table 2 illustrates our growth in numbers of papers submitted to the Society, showing in five-year blocks the average number submitted each year. From 1929-1933 there was an average of 18 papers submitted yearly; from 1934-1938 the average was 29; from 1939-1943 it was 43; from 1944-1948 it was 37; from 1949-1953 it was 75, and from 1954-1958 the papers averaged 128 each year.

This growth represents many factors, among which have been increasing interest in prevention of disease, the appearance of antibiotics, the growth of our population, and the interest of our nation and government in research into the cause and prevention of disease and abnormality. Our growth has been inevitable and we cannot look back.

I feel that I have offered ample proof that our Society has attained the status of adulthood. Are there any signs of weakness in this adult which point to future difficulties? Our most striking weakness is the very diversity of our interests. When

TABLE 2.—*Total Number of Papers Submitted to Society for Pediatric Research (Average per Year)*

| 5-Year Periods | 1929-1933 | 1934-1938 | 1939-1943 | 1944-1948 | 1949-1953 | 1954-1958 |
|---|---|---|---|---|---|---|
| Average No. of Papers per Year | 18 | 29 | 43 | 37 | 75 | 128 |

the Society was founded and 63% of the papers submitted were on metabolic problems we were a close-knit group. Now this is no longer so, and we are split into smaller groups each with its own particular field. We have felt increasing pressure from these groups, each suggesting that the plenary sessions be devoted to a whole morning on cardiology, an afternoon on endocrinology, and so forth. To date we have largely resisted this pressure but have yielded to the extent that one evening is devoted to simultaneous meetings of the subspecialty groups. I feel strongly that we must continue to present a diverse and broad program in our plenary sessions. Though we are gathered together because of our interest in research, we are not solely investigators but we are also teachers and must continue to be aware of the work which is conducted in fields outside our own domains. We must hold together not because of our own interest in virology or cardiology or nephrosis but because of our interest in children. We have witnessed the appearance of regional societies for pediatric research and have given these new organizations our blessings and good wishes, but as a group of physicians vitally concerned with research into the health and welfare of children we must not become overly specialized or separated into districts. It is essential for the growth of research into the problems of children that we continue to function as a national society with broad, undivided interests.

In closing, I wish us a happy birthday and many more to come.

Dr. Joseph Johnston supplied the photographs; Dr. James Gamble and Dr. Charles McKhann furnished early material, much of which could not be included in an address before a society whose scientific papers are limited to 10 minutes, rendering a lengthy presidential address not only inappropriate but unkind.

818 Harrison Ave. (18).

# Transactions of the Society for Pediatric Research

*Twenty-Ninth Annual Meeting*

**Buck Hill Falls, Pa., May 8-9, 1959**

**SYDNEY S. GELLIS, President; MARGARET H. D. SMITH, M.D., Vice President; CLARK D. WEST, M.D., Secretary-Treasurer**

## SCIENTIFIC PROGRAM

### First Plenary Session

1. **Presidential Address**
   Sydney S. Gellis
2. **Studies of Red Cell Survival in Early Infancy**
   Eugene Kaplan (71)
3. **Apparatus for Establishment of Separate Extracorporeal Fetal and Maternal Circulations in the Human Placenta**
   Kermit E. Krantz * and T. C. Panos (170)
4. **Effects of Human Growth Hormone and of Modified Bovine Growth Hormone**
   John J. Hutchings,* Roberto F. Escamilla,* William C. Deamer, and C. H. Li * (Introduced by Henry B. Bruyn) (67)
5. **The Production of 19S γ-Globulin Antibody by Neonatal Infants in Response to Typhoid Immunization**
   Richard T. Smith, John James,* Donald V. Eitzman,* and Billievelyn Miller * (127)
6. **Remarks on Glucose Transport Across the Liver Cell, and on Liver Hexokinase**
   J. W. Appelboom * and William A. Brodsky (157)
7. **Enzyme Studies in Renal Tubular Acidosis**
   Sumner J. Yaffe,* John M. Craig, and Francis X. Fellers * (154)
8. **Immunization of Children with a Live Attenuated Measles Virus**
   Samuel L. Katz * and John F. Enders * (Introduced by Sidney Kibrick) (73)
9. **A Hemagglutination Test for Staphylococcal Disease**
   Alfred L. Florman, Jeanne L. Scoma,* Helen Zepp,* and Eugene Ainbender * (47)
10. **A New Abnormality of Tryptophan Metabolism in Phenylketonuria**
    Samuel P. Bessman and Keiya Tada * (13)

* By invitation. Number in parentheses is abstract number.

### Second Plenary Session

11. **Clinical Observations on the Cardiopulmonary Status of Infants with Hyaline Membrane Disease**
    J. E. Drorbaugh,* A. J. Rudolph,* P. A. M. Auld,* R. B. Cherry,* and C. A. Smith (161)
12. **Studies on the Circulation of Infants with Hyaline Membrane Disease**
    A. M. Rudolph,. P. A. M. Auld,* J. E. Drorbaugh,* A. J. Rudolph,* A. S. Nadas, and C. A. Smith (111)
13. **Oxygen Affinity and Capacity of Red Cells from Normal and Erythroblastotic Newborns**
    Abraham Abrahamov,* Clement A. Smith, and Louis K. Diamond (1)
14. **Bilirubin Metabolism in the Fetus**
    R. Schmid,* S. Buckingham,* L. Hammaker,* and G. Medenilla * (Introduced by Sydney S. Gellis) (113)
15. **Potassium, Mitochondria, and Mercury Compounds**
    J. L. Gamble Jr., R. L. Scott,* and O. Reiss * (51)
16. **The Persistence of Virus in the Environment of Patients with Kaposi's Varicelliform Eruption**
    Sidney Kibrick (77)
17. **Comparison of the in Vitro Metabolism of Tetrahydrocortisone in the Fetal, Newborn, Pregnant, and Nonpregnant Adult Rat Liver**
    Robert E. Greenberg * and Lytt I. Gardner (165)
18. **Studies of Cells Cultivated in Vitro from Persons with Genetic Diseases**
    Arthur Robinson * and Theodore Puck * (Introduced by Henry K. Silver) (110)
19. **Changes in the Blood Volume of Infants in the First Hours of Life**
    Thomas R. C. Sisson (120)
20. **Urinary Ketosteroid Sulfates and Glucuronides**
    J. Darrel Smith * (Introduced by Walter R. Eberlein) (125)

## Third Plenary Session

21. **Pickwickian Syndrome in Children**
Glen G. Cayler,* Harris D. Riley Jr.,* and James Mays* (Introduced by Alexander S. Nadas) (158)

22. **Evidence for a Bond Between RNA and Glycogen Induced by Cortisone in Rat Liver**
Charles U. Lowe (173)

23. **Mechanisms of Therapeusis in Hyperkalemic Familial Periodic Paralysis**
R. Klein and T. Egan* (80)

24. **Role of PAP (Eaton-Liu) Virus in Childhood Respiratory Disease**
M. K. Cook,* R. M. Chanock, R. H. Parrott, and R. J. Huebner* (31)

25. **Proteinuria in Health and Disease**
R. J. Slater, B. Brown,* D. Mills,* and P. Azzopardi* (123)

26. **The Relationship of Amino Acids to Idiopathic Hypoglycemia**
W. A. Cochrane* and F. Moya* (Introduced by R. Slater) (29)

27. **Simultaneous Right and Left Heart Catheterization Employing Retrograde Arterial Catheterization in the Investigation of Congenital Cardiovascular Malformations of Infants and Children**
Peter Vlad* and Edward C. Lambert* (Introduced by Mitchell I. Rubin) (146)

28. **A Factor in Normal Human Plasma Which Stimulates Platelet Production: Chronic Thrombocytopenic Purpura Due to Its Deficiency**
Irving Schulman, Mila Pierce,* Abby Lukens,* Zinet Currimbhoy,* and Eleanor Fort* (114)

29. **Survival of Skin Homografts Following Exchange Transfusion for Neonatal Jaundice**
R. Fowler Jr.,* W. K. Schubert,* and C. D. West (49)

30. **Viral Infection: A Possible Cause of Sudden, Unexpected Death in Infants**
Eli Gold, David Carver,* Hannelore Heineberg,* and Lester Adelson* (55)

## Special Sessions

*Section on Hematology.* North Lounge
Moderator: Irving Schulman

31. **Bilirubin Inhibition of Heme Biosynthesis**
Robert F. Labbe,* Merlin R. Zaske,* and Robert A. Aldrich (82)

32. **Infantile Pyknocytosis, a Common Erythrocyte Abnormality of the First Trimester**
Philomena Tuffy,* Audrey K. Brown, and Wolf W. Zuelzer (139)

33. **Treatment of Terminal Leukemic Relapse by Total-Body X-Irradiation and Intravenous Infusion of Autologous Bone Marrow Stored During Remission**
Joseph J. McGovern Jr,* Paul S. Russell,* Leonard Atkins,* and Edward W. Webster* (Introduced by Nathan B. Talbot) (97)

34. **An Electrophoretic Method for the Detection of Leukocyte Antibodies**
S. L. Leikin* and Gloria D. Eng* (Introduced by Robert H. Parrot) (84)

35. **Erythrocytic Glucose-6-Phosphate-Dehydrogenase Activity and Anemia Due to Infection**
Senih Fikrig* and R. Janet Watson (46)

36. **Glucose-6-Phosphate Dehydrogenase Activity in Patients with Various Abnormal Hemoglobins**
Jean Naylor,* Ira Rosenthal,* Aaron Grossman,* Irving Schulman, and David Yi-Yung Hsia (99)

37. **Enzyme Deficiency in Erythrocytes in Congenital Nonspherocytic Hemolytic Anemia**
Nasrollah T. Shahidi* and Louis K. Diamond (117)

38. **The Comparative Value of Intramuscular Versus Orally Administered Iron in the Prevention of the Late Anemia of Prematurity**
A. L. Sitarz,* J. A. Wolff, and F. H. Von Hofe* (121)

*Section on Cardiopulmonary Disease.*—East Room
Moderator: J. F. Dammann Jr.

39. **The Heart Sounds in the Newly Born Infant**
M. Braudo* and R. D. Rowe (22)

40. **Electrocardiographic Evaluation of Pulmonic Stenosis**
Mary Allen Engle, Tomiko Ito,* Daniel S. Lukas,* and Henry P. Goldberg* (44)

41. **Reversibility of Pulmonary Hypertension Following Closure of Ventricular Septal Defects**
Paul Adams Jr., Ray C. Anderson, C. Walton Lillehei,* and Nicolaas Meyne* (2)

42. **Prognostic Value of Serial Electrocardiography in Isolated Ventricular Septal Defects**
W. M. Thompson Jr.,* Omar Sosa,* and J. F. Dammann Jr. (138)

43. **Total Anomalous Pulmonary Venous Return: Infradiaphragmatic Type, Clinical Manifestations**
Blanca Smith,* Thomas R. Frye,* and W. A. Newton Jr.* (Introduced by Warren E. Wheeler) (124)

* By invitation. Number in parentheses is abstract number.

44. **The Carbon Dioxide Spirometer: A New Instrument for the Rapid Evaluation of the Circulation**
John J. Osborn (104)

45. **Vectorial Analysis of the Electrocardiogram of the Newborn and Premature in Health and in Disease**
Carlos Vallbona* and Murdina M. Desmond (141)

46. **Direct Plasma Oxygen Tension Determination in Infants and Adults in Air and in Modified Atmospheres**
Bruce D. Graham, Steve T. Koeff,* M. U. Tsao,* C. Sloan,* and James L. Wilson (56)

*Section on Virology.*—Convention Lodge
Moderator: Frederick C. Robbins

47. **Antibody-Resistant Vaccinia Necrosum Cured by Passive Transfer Sensitization**
C. Henry Kempe and Alton J. Morris* (75)

48. **Prevalence of Poliovirus Infection in a Partially Immunized School Population**
Paul F. Wehrle, Ory Carbonaro,* and Ralph Reichert* (148)

49. **The Contribution of Detectable Viruses to the Croup Syndrome**
Robert H. Parrott, Robert M. Chanock, and Andrew J. Vargosko* (105)

50. **Experience with Inoculation of Increased Amounts of Poliomyelitis Vaccine**
Samuel Baron* and Eugene V. Barnett* (Introduced by Robert M. Chanock) (8)

51. **Poliovirus Neutralizing Substances in the Feces of Paralytic Poliomyelitis Patients**
Murray M. Lipton* and Alex J. Steigman (88)

52. **Respiratory Illness in Children Simultaneously Infected with Hemadsorption Virus Type 1 and a Newly Recognized HeLa Enterovirus (HE)**
Henry G. Cramblett, Julius A. Kasel,* and John P. Utz* (35)

53. **Infectious Ribonucleic Acid Derived from Enteroviruses**
Katherine Sprunt,* Winifred M. Redman,* and Hattie E. Alexander (129)

54. **Properties of Infectious Ribonucleic Acid from Highly Purified Poliovirus Tested in Human Cell Monolayers**
Gebhard Koch,* Susan Koenig,* Olga Van Damme,* and Hattie E. Alexander (81)

*Section on Metabolism.*—Auditorium
Moderator: William A. Brodsky

55. **Enzymatic Detection of the Galactosemic Trait**
H. N. Kirkman* (Introduced by Victor A. Najjar) (78)

56. **Excretion of Phenolic Compounds by Premature Infants**
Moises Behar,* Lewis A. Barness, and Paul György* (9)

57. **Dehydration, Starvation, and Trauma: Their Effects on Body Composition**
William B. Weil Jr. and Anne Taylor* (149)

58. **The Distribution of Water Loss of Hypernatremic Dehydration**
Malcolm Holliday and James Pitcavage* (65)

59. **Disturbances of Sodium and Water Homeostasis in Hypernatremic Infants**
Gerald A. Kerrigan (76)

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### 1. Oxygen Affinity and Capacity of Red Cells from Normal and Erythroblastotic Newborns

ABRAHAM ABRAHAMOV,* CLEMENT A. SMITH, and LOUIS K. DIAMOND, Harvard

Solutions of fetal and adult hemoglobin have similar oxygen affinities once their other solutes are equalized by dialysis (Allen, W., and Smith, J.: *Biol. Chem.* 203:81, 1953). To test whether composition of the plasma environment affects the oxygen affinity of hemoglobin in fetal and adult erythrocytes, oxygen dissociation curves were constructed for corpuscles of adults in plasma of newborns after exchange transfusion in 10 infants with erythroblastosis. In other studies, cells of newborn infants were suspended in vitro in plasma from adults. The results indicated that the red cells of adults retain their expected oxygen affinity in the plasma of newborn infants and vice versa.

The oxygen carrying capacity of 1 gm. of hemoglobin was found to be normal (mean, 1.337 ml.) in blood from healthy newborn infants, contrary to the report of Swierczewski and Minkowski (*Études néo-natal.* 5:11, 1956). On the other hand, the oxygen capacity of hemoglobin of infants with erythroblastosis was found to be 15%-31% below the expected normal.

In the second part of these studies, the possible explanation of this finding was sought. To test the effect of anti-Rh serum on the oxygen capacity of the Rh-positive red cells, 30 Group O, Rh-positive blood samples were obtained. One-half of each was used as a control. The plasma of the other half was removed and the erythrocytes suspended in standard anti-Rh (anti-D) serum in their original proportions. These cell suspensions were then held at 37 and 4 C for different periods of time, between 1 and 24 hours, after which oxygen capacity and direct Coomb's test were determined. The Rh-positive red cells retained their normal oxygen capacity for about 12 hours of incubation with anti-Rh serum at 37 C. Longer incubation was associated with a significant and progressive reduction of oxygen capacity. No changes occurred in any of the control samples. The Rh-positive erythrocytes suspended in anti-Rh serum at 4 C for 1-24 hours showed no reduction of oxygen capacity. Therefore, the combination of Rh-positive red cells and anti-Rh antibodies alone appears to be insufficient for reducing oxygen capacity immediately. A temperature above a certain minimum and a time interval of between 12 and 24 hours are required.

These observations may have clinical implications with respect to the development of various signs and symptoms in the infant with erythroblastosis fetalis due to maternal antibody coating of fetal red cells.

#### Discussion

DR. AUDREY BROWN, Detroit: This is certainly a beautiful piece of work. I would like to ask is there a difference in oxygen affinity between ABO erythroblastotics and Rh erythroblastotics?

Also in your studies with bilirubin, were you able to detect any effect of unconjugated bilirubin in the presence of antibody; was there perhaps an enhancing effect of unconjugated bilirubin on the antibody on the cell?

And thirdly, was there any correlation with the degree of reticulocytosis present in the blood of these infants?

DR. IRVING SCHULMAN, Chicago: I was also interested in studies of ABO incompatibility, but from a slightly different point of view. It has been shown in animals that oxygen affinity seems to be dependent upon the thickness of the red cell. In ABO incompatibility, as opposed to Rh incompatibility, spherocytosis is known to occur. Spherocytes, being abnormally thick red cells, produce a shift to the left in the oxygen disassociation curve to shift to the right. Have the various changes in red cell morphology been evaluated in regard to your findings?

DR. SYDNEY SEGAL, Vancouver, B. C., Canada: This paper will stimulate many new studies. Have the pH and $CO_2$ been observed and controlled in order to rule out the effects of these factors on oxygen affinity? Secondly, in line with Dr. Graham's exposition of the importance of oxygen tension measurements, not only to determine the ability of red cells to take up oxygen, but also to evaluate the gradient which forces it to deliver this gas to the tissues, has Dr. Abrahamov constructed any dissociation curves on these samples to show whether there may be a difference of inflection as well as a shift of their position?

DR. IRVING SCHULMAN, Chicago: Russian workers have implicated the plasma magnesium level as influencing oxygen disassociation. Has this been measured in your studies?

DR. DANIEL C. DARROW, Kansas City, Kan.: I wonder if the method could be modified to measure rate instead of the end-point. It's the rate which would be important here.

DR. ABRAHAMOV: I am sorry that in answer to most of the questions I will have to say "no." But in response to Dr. Brown's question, we intend to examine the ABO incompatibilities as well as the Rh. We have not done this up till now.

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The abstracts are arranged in two series, each alphabetized with respect to the first author's name. The first includes Abstracts 1 to 155, the second Abstracts 156 to 179. The abstracts of papers read in the Plenary Sessions are each followed by discussion.

* By invitation.

We haven't measured the reticulocytes in those patients and we haven't related them to the oxygen capacity or to the amount of glycolysis. Although there is some indication that high amounts of reticulocytes in the blood have increased amounts of glycolysis, we still might say that there is a very significant reduction of the glycolysis. We might have expected the opposite, that the glycolytic rate was increased.

To answer Dr. Schulman's question, we have not examined the ABO incompatibilities and we haven't looked for target cells; neither did we examine for magnesium or other heavy metals.

In response to Dr. Segal's question about pH and $CO_2$, we have examined both the pH and $CO_2$ of those bloods and all were in the same range.

### 2. Reversibility of Pulmonary Hypertension Following Closure of Ventricular Septal Defects

PAUL ADAMS JR., RAY C. ANDERSON, C. WALTON LILLEHEI,* and NICOLAAS MEYNE,* Minnesota

It has been theorized frequently and recently demonstrated by Heath et al. at postmortem examinations that pulmonary hypertension associated with organic pulmonary pathology consisting of intimal proliferation of small arterioles associated with congenital heart disease is not immediately reversible following closure of the defect. Immediate falls in pulmonary artery pressures toward normal in patients showing less severe pulmonary pathology has been interpreted as resulting from the elimination of the large left to right shunt. Whether further regression of pulmonary hypertension would occur in the months after surgery remained unanswered.

This study is based upon 46 patients who survived closure of their ventricular septal defect and subsequently have been shown to have complete closure of their defect upon recatheterization an average of one year later. Results indicate that the fall in pressures observed even after one year is related only to the elimination of the large left-to-right shunt, in that the calculated total pulmonary resistance remained unchanged or had a tendency to rise above preoperative values.

For purposes of comparison by retrospection the postsurgical catheterization data were divided into three groups according to the decrease of pulmonary pressures toward normal. The group of patients with the highest residual mean pulmonary artery pressures averaged about three times greater pressures than the group with the lowest residual mean pulmonary artery pressures, which were close to normal values.

Comparison of the preoperative data of these two groups showed that the poor response group had greater mean pulmonary artery pressures, greater total pulmonary resistance, and slightly less pulmonary flow than in the good-response or "normal" group. The median age at the time of operation in the poor response group was 5 years, compared to less than 1 year in the good response group. The growth and development was normal in the poor-response (older age) group and significantly poorer in the good-response group.

Lung biopsy specimens were available in about one-half of the group. Significant correlations are limited by the small numbers and the fact that early in our surgical experience the number of survivors with severe pulmonary pathologic changes were rare.

Changes in the electrocardiogram, roentgen studies, and auscultatory findings do not appear to have any reliable value in the prediction of the best surgical result.

On the basis of this study it appears reversibility of pulmonary hypertension following closure of the ventricular septal defect results from the reduction of the large pulmonary flow up until one year following surgery. Whether in the years to follow, regression, stabilization, or gradual premature progression might occur must await a longer follow-up period.

### 3. Further Hemodynamic Observations in Children with Homozygous Hemoglobin S Disease

LORIN E. AINGER,* HENRY H. LONG,* JAMES N. ETTELDORF, and CARL B. FARRELL,* University of Tennessee

Cardiac manifestations form a prominent but relatively little-studied aspect of the clinical picture of sickle-cell anemia. Cardiomegaly, with or without congestive heart failure, loud systolic murmurs, and electrocardiographic evidence of left ventricular hypertrophy are observed with frequency in infants, children, and adults with this disease.

Right heart catheterization was performed in eight patients with homozygous hemoglobin-S disease. There were five females and three males, ranging in age from 9 to 18 years. Intracardiac and pulmonary arterial pressures were measured and cardiac outputs were determined by the Fick principle.

Total plasma and blood volumes were determined by the Evan's blue dye (T-1824) dilution technique. In the proceedings of a previous meeting of this Society (1955), this group reported the results of Evans blue dye determinations of plasma and total blood volumes in normal children and children with S-S disease and nutritional anemia, with use of a six-minute equilibration period. This method yielded values lower than those obtained by the more conventionally used 10-minute equilibration period. There is reason to believe that

* By invitation.

the six-minute equilibration period yields a truer value of total plasma and total blood volumes. In unpublished data, Overman et al. have demonstrated that dye appears in the thoracic duct within nine minutes. It is obvious, therefore, that equilibration periods greater than this would tend to yield abnormally high values and would suggest a need for a reevaluation of this technique and of the previously published data reported in normal and abnormal states. In addition, Freeman, using $Cr^{51}$-tagged red blood cells, has shown that equilibration periods of only three minutes give more accurate results than longer periods.

Significant desaturation of the peripheral arterial blood was observed in four of the eight patients. These values were 81%, 81%, 83%, and 83%. This confirms similar observations in adults made by Sproule, Halden, and Miller. Intracardiac and pulmonary arterial pressures and pulmonary vascular resistances were normal. Total blood volumes were increased moderately in three of the six patients in whom these determinations were made; this increase was due primarily to an increase in the total plasma volume. Cardiac outputs were increased uniformly. Values for the cardiac index were: 6.6, 7.6, 7.7, 5.4, 5.2, 8.9 and 9.4 liters per minute. In general, there was a good correlation between the total blood volume and the cardiac output.

From these studies the cardiovascular manifestations of sickle-cell anemia may be ascribed to (1) the chronic anemia, per se; (2) the increased cardiac output; and (3) the possible existence of a right-to-left shunt within the pulmonary parenchyma producing peripheral arterial desaturation, as suggested by Sproule, Halden, and Miller.

### 4. The Effect of Saccharolactone on Bilirubin and Bilirubin Excretion in Newborn Infants

WOLFGANG K. ANKE,* ROBERT FENICHEL,* and LEWIS A. BARNESS, University of Pennsylvania

Bilirubin glucuronide formation in the newborn infant is low. In hemolytic states in newborn infants the decreased glucuronide formation may lead to severe disease.

In vitro studies reveal that bilirubin glucuronide which is formed may be broken down with $\beta$-glucuronidase to give unconjugated (indirect) bilirubin. Since $\beta$-glucuronidase is elevated in newborns, a $\beta$-glucuronidase inhibitor was studied.

Rat-liver homogenates were incubated with $\beta$-glucuronidase and 1,4-saccharolactone, an inhibitor of $\beta$-glucuronidase. When $\beta$-glucuronidase alone was added to the rat liver slices, decreased formation of direct bilirubin was noted. However, when saccharolactone was added to the mixture, increased amounts of direct bilirubin were noted.

Therefore, normal 2-day-old babies with physiologic jaundice were fed by gastric tube 3 gm. of saccharolactone over a period of two to three hours. Total bilirubin in the serum remained constant or decreased.

### 5. Pseudomonas Infection in Children

LYAL D. ASAY* and RICHARD KOCH,*
University of Southern California
Introduced by Robert Ward

Pseudomonas infections as documented by positive cultures at the Los Angeles Childrens Hospital have increased yearly from a total of 8 in 1952 to 152 in 1957. The number of deaths associated with pseudomonas infections increased from 2 in 1952 to 21 in 1957, thus indicating that this organism now constitutes a problem of some magnitude.

A review of the fatal cases during 1957 reveals that all were ill with some other primary disease. In order of frequency these were abdominal anomalies; respiratory anomalies, including tracheoesophageal fistula; gastroenteritis; meningomyelocele; malignancy; fibrocystic disease of the pancreas, and obstructive uropathy. Fifty per cent were under one month of age at the time of death. An additional 25% were under 6 months of age and only four children, or 13%, were beyond the age of 3 years. Two of these four were ill with leukemia and the other two with fibrocystic disease.

Pseudomonas was cultured from 75 patients with appendicitis, wound infections, otitis media, abscesses, and urinary tract infections. These patients were treated primarily by surgery including adequate drainage and all survived. The organism was cultured from the upper respiratory tract of 29 patients with chronic pulmonary or cardiopulmonary diseases, including 6 with tracheoesophageal fistulas. Twenty-two of the twenty-nine patients survived. Only 4 of 12 patients with congenital abdominal anomalies who developed pseudomonas infections following surgery survived. Two of three patients with Pseudomonas meningitis associated with either meningomyelocele or following surgery for hydrocephalus survived. Four of seven patients with Pseudomonas enteritis survived, as did all seven of those with extensive burns. One of the latter had associated Pseudomonas sepsis with multiple abscesses.

Antibiotic sensitivity studies with use of the agar plate antibiotic-impregnated disk technique revealed a marked decrease in sensitivity of the organism over the years to all antibiotics tested except polymyxin. In 1957 the organisms sensitive to each antibiotic were as follows: polymyxin 92%, oxytetracycline 42%, tetracycline 33%, streptomycin 21%, and chloramphenicol 5%.

* By invitation.

Polymyxin alone, however, was not usually found to be effective in treating serious Pseudomonas infections. The addition of oxytetracycline, tetracycline, or streptomycin to the therapy of sepsis, pneumonia, meningitis, or spreading abscesses was, however, effective when the diagnosis was suspected early.

### 6. The Role of Surface Forces in Atelectasis of Prematurity and Hyaline Membrane Disease

MARY ELLEN AVERY,* Harvard
Introduced by Clement A. Smith

The work of Clements has indicated that the alveoli of adult animal lungs are lined by a layer, presumably protein, which is capable of having a very low tension at the gas-liquid interface. Such a lining layer would contribute to the stability of the alveoli by reducing their tendency to collapse at low lung volumes.

Small premature infants, and infants with hyaline membrane disease, are predisposed to atelectasis. Because of the possibility that they might have a deficient lung-lining layer, the surface tension of washings of the tracheal-bronchial tree, as well as extracts of their lungs, was compared with similar washings and extracts of lungs of older infants and children.

Measurements of surface tension, made on a Wilhelmy film balance, with the method of Clements (*Proc. Soc. Exper. Biol. & Med.* 95: 170, 1957), were strikingly different in the two groups. Lungs of 10 infants with a birth weight under 1,100-1,200 gm. and 11 infants with hyaline membrane disease regardless of birth weight had tensions of about 25-30 dynes per centimeter when the surface area was decreased. In contrast, lungs of 38 infants over 1,200 gm. and older children had tensions under 20 dynes per centimeter at small surface areas, and usually as low as 5-10 dynes per centimeter.

These findings suggest that the predisposition of immature infants and infants with hyaline membrane disease to atelectasis may relate to this deficiency in the alveolar lining.

### 7. Phage-Altered Characteristics of Atypical Mycobacteria and Nocardiae

EDWARD N. AZAROWICZ,* Johns Hopkins
Introduced by Robert E. Cooke

An atypical organism, named Nocardia intracellularis, was reported by Cuttino and McCabe as a new species of branching acid-fast pathogenic organism. This new Nocardia has some characteristics of Mycobacterium tuberculosis, and some question concerning its identification arose. This organism was used to prepare one of five skin-testing antigens to test for tuberculin sensitivity in India and the Philippines by Edwards and Palmer under a United States Public Health Service Program. They found widespread sensitivity to this antigen and stated their conclusion that nonspecific sensitivity is produced by a variety of organisms some of which may not even be mycobacteria. This and many other examples suggested an overlap between the two genera Mycobacterium and Nocardia. As a corollary, it was found that some atypical mycobacteria resemble atypical nocardiae; and it was also found that some atypical strains of both genera were lysogenic.

All lysogenic nocardiae were found to be smooth and fast-growing, showed little or no branching, and had yellow-orange pigment or were ivory-colored, somewhat resembling chromogenic and Battey type mycobacteria. Indeed, our lysogenic Nocardia globerula can also be recognized under the name of Mycobacterium rhodchrous.

Transduction has been observed with two strains of N. globerula no. 39 and 41, which are very similar except for two points of difference; no. 39 is lysogenic and cannot utilize citrate as sole carbon source, whereas no. 41 is nonlysogenic, can utilize citrate, and is the propagating strain for the phage carried by no. 39. The phage can be transferred from no. 39 (citrate-negative) to 41 (citrate-positive) and then back to no. 39, which now becomes positive for citrate utilization. Other organisms are being studied to determine whether transduction by phage can be a relatively common occurrence. Transduction can, conceivably, account for some variations and the "atypical" state of actinomycetes.

The phenomenon of clumping of cells by phage into one large viscous mass has been observed. M. tuberculosis ATCC 607 is clumped by mycobacteriophage 1M/M2. The cells without phage grow well dispersed in broth, but in the presence of phage the large, single clump cannot be dispersed by shaking. This clump is, however, dispersed instantly upon the addition of deoxyribonuclease (DNA). Trypsin does not disperse the clump. This suggests an alteration of a growth characteristic by extracellular DNA as a result of phage infection.

One strain of Nocardia asteroids which produces abundant branching mycelium was partially lysed, breaking up the mycelium into short bacillary forms which have never reverted to branching even after several years. This bacillary, fast-growing form which still has the orange pigment of the parent N. asteroides now resembles an atypical chromogenic Mycobacterium.

It is anticipated that whenever a phage-induced characteristic can be recognized as such, the atypical organism can be given the generic or specific name of a prototype strain. It is hoped that by describing the limits of experimentally induced phage modifications all variations for a given species may eventually be described.

* By invitation.

### 8. Experience with Inoculation of Increased Amounts of Poliomyelitis Vaccine

SAMUEL BARON * and EUGENE V. BARNETT,*
Division of Biologic Standards, N. I. H., Bethesda, Md.
Introduced by Robert M. Chanock

A previous study investigated a method for rapid immunization with currently available poliomyelitis vaccine. A single inoculation of 10 ml. of poliomyelitis vaccine was shown to result in early development of antibody when compared with a single 1 ml. inoculation. These observations were made using a limited number of non-immune persons who were inoculated with vaccine lots that were somewhat above the median potency of other 1958 vaccine lots (Baron, et al., *New Eng. J. of Med.*, in press.). Such a method might provide protection for those persons who were not immune prior to an epidemic season.

The purpose of the present study was to (1) determine the effect of graded amounts of a vaccine lot containing a minimum amount of antigen, given in a single inoculation, on the antibody response of non-immune persons, (2) evaluate the antibody response of partially immune persons to increased amounts of the same vaccine lot, and (3) gain further experience regarding the safety of inoculating increased amounts of currently available poliomyelitis vaccine. The antibody response to poliomyelitis vaccine was again shown to be dependent upon the amount of antigen inoculated. The response was shown to be influenced by the presence of preexisting naturally acquired heterologous and homologous poliovirus antibodies. Ten milliliters of a minimum-potency vaccine lot elicited a greater antibody response than one milliliter. This response was not as high as that reported previously when 10 ml. of a vaccine lot containing greater amounts of antigen was used. One hundred twelve persons, 80% of whom were children under 5 years of age, have received 10 ml. of three different vaccine lots, and there have been no untoward effects.

### 9. Excretion of Phenolic Compounds by Premature Infants

MOISES BEHAR,* LEWIS A. BARNESS, and PAUL GYÖRGY,* University of Pennsylvania, Philadelphia General Hospital and INCAP.

The source of urinary phenols in infants is unknown but is generally believed to be related to ingestion of tyrosine-containing foods and due to degradation in the intestine or metabolic degradation in the body. Excretion in the premature would depend also on the ability of the kidney to clear phenolic substances.

Urinary excretion of phenols in children 17 to 35 days of age (*J. Pediat.* 51:40, 1957) has been found to decrease with increasing lactose in the diet.

Urinary phenols, both free and conjugated, were measured in premature infants fed low- and high-protein diets. The effects of antibiotics, age, lactose, and vitamins were noted. It was found that phenols increase with increasing age. The youngest infants excrete the smallest amounts of both free and conjugated phenols regardless of diet. There was a direct relation between the total amount of phenols excreted and the volume of urine excreted. Concentration of phenolic substances in the urine remained practically constant.

Phenolic excretion was found to be closely related to the total creatinine excreted by the premature. There was a close correlation also between the phenolic excretion and the weight of the infant.

No relation was noted to intake of vitamins A, C, and D. Ingestion of chlortetracycline (Aureomycin) was found not to change or to increase slightly the excretion of phenolic substances. There was a slight increase in phenolic excretion with high-protein diet compared with low-protein diet.

Prematures of any age are able to conjugate about one-third to one-half of the total phenols excreted.

### 10. The de Toni-Fanconi Syndrome in Childhood

JAY BERNSTEIN,* RUBEN MEYER,* and FLOSSIE COHEN,* Children's Hospital of Michigan
Introduced by Paul V. Woolley Jr.

If the de Toni-Fanconi syndrome is defined as comprising hypophosphatemic rickets in association with increased renal excretion of glucose and amino acids, several important observations may be drawn from a study of reported cases: 1. In childhood most, but not all cases are associated with cystine-storage disease, and, conversely, not all cases of the latter are complicated by this syndrome. 2. Functional data indicate that an abnormality in the proximal portion of the renal tubule is responsible for the syndrome, but in a significant number of cases the kidneys have been histologically normal despite the presence of generalized cystinosis and a fully developed syndrome. It may therefore be surmised that the renal tubular malfunction is secondary and may be caused by or associated with a number of conditions in addition to cystinosis and that the resultant syndrome is the same no matter what the cause. The associated states other than cystinosis which have been described in children include hepatic cirrhosis, the nephrotic syndrome, heavy-metal poisoning, von Gierke's disease, and familial congenital abnormalities. Furthermore, the functional alteration in the renal tubule may in at least the cases of cystinosis occur independently of structural alteration.

* By invitation.

Five cases, three with cystine-storage disease and two with hepatic cirrhosis, have been included in the present study. Of the former, two were siblings, and although the older seemed to have a complete syndrome, rickets and dwarfism were not present in the younger when he died of apparent renal failure at the age of 17 months. The third case of cystinosis had at the age of 1½ years rickets which was subsequently controlled with large doses of vitamin D. His renal function has remained well preserved, and now, at the age of 16 years, there is no evidence of impending deterioration. It may be pertinent that his serum potassium levels have always been normal. Of the two cases of cirrhosis, one had evidence of active hepatitis found on biopsy, but in neither was there evidence of cystine-storage disease.

These cases strengthen the observation that the full syndrome of hypophosphatemic rickets, aminoaciduria and glucosuria is not a constant companion of cystine-storage disease, and to that it may be added that inexorable renal failure is not the inevitable fate of those afflicted with the syndrome. This syndrome may theoretically accompany any condition which produces less than total injury to the proximal portion of the renal tubule, thereby impairing the reabsorptive mechanisms of glucose, phosphate, and amino acids, and circumstantial evidence may be adduced to show that the morphologic changes and possibly the progressive deterioration of renal function may be in part due to chronic hypokaliemia.

### 11. A Histologic Appraisal of the Kidney in Sickle-Cell Anemia

JAY BERNSTEIN * and CHARLES F. WHITTEN,*
Children's Hospital of Michigan
and Detroit Receiving Hospital
Introduced by Wolf W. Zuelzer

In children with sickle-cell anemia, the following major pathophysiologic findings related to the kidney have been described: Virtually all children with sickle-cell anemia have a defect in concentrating ability during water deprivation; many have an increase in glomerular filtration rate and maximal excretory capacity for PAH; an occasional child has gross unilateral hematuria, and more rarely the nephrotic syndrome and renal failure have occurred. Because of a paucity of reported data in children, a systematic histopathologic appraisal of the kidney has been undertaken in 15 autopsied cases of sickle-cell anemia, including one diagnosed as sickle-cell thalassemia disease, in an effort to correlate anatomic alterations with these observations.

The most striking findings were in the glomeruli of the inner third of the cortex—the juxtamedullary glomeruli. Actual glomerular enlargement, in five cases estimated by semiquantitative methods at 30% more than normal, was present. This enlargement appeared to be the result of increased vascularity and cellularity rather than congestion or simple capillary ectasia. An additional observation was focal glomerular hemorrhage without actual thrombus formation. This disruption of the glomerular tuft, with exudation of blood into Bowman's space and the tubule, seemed to be the first step in fibrosis and scarring which progressively involved lobules of an entire glomeruli. Glomerulosclerosis was more evident than usual in all the cases, and in one case the process had progressed to widespread glomerular fibrosis and obliteration, which was of sufficient magnitude to account for clinical renal impairment.

An incidence of active tubular necrosis was confirmed in three cases, and in two others regeneration and cicatrization were taken as evidence of a healing process. In 11 cases hemosiderin pigment was present in the tubular epithelium, occasionally concentrated in tubules which had been distorted by scarring.

Alterations in the medullary papillae were encountered with surprisingly high frequency. Edema, telangiectasia, and an increased amount of connective tissue were seen in nine cases. The stroma had a myxomatous, cellular appearance, and there were variable fibrous scarring, a decrease in the number of tubules, constricted epithelial nests, and tubular retention cysts. In at least one case there appeared to have been a loss of substance with blunting of pyramids.

The tubular alterations and papillary scarring may be reflected in the impaired ability to concentrate urine. The enlargement of the juxtamedullary glomeruli possibly provides an anatomic basis for the previously unexplained increased filtration rate seen in some children, and the progressive glomerular scarring offers a clue to the pathogenesis of the decline in filtration rate in older patients and the occasional reports of renal failure in children with sickle-cell anemia.

### 12. Serum Protein Patterns in Acute Poststreptococcal Glomerulonephritis

STANLEY H. BERNSTEIN,*
Long Island Jewish Hospital
Introduced by Samuel Karelitz

Conflicting data regarding the changes in serum protein patterns during the course of acute glomerulonephritis may be related to the multiplicity of etiologic agents producing the clinical syndrome.

Twenty-five patients with fairly uniform clinical and laboratory characteristics of acute nephritis were studied. All patients had hematuria, albuminuria, edema, and elevated erythrocyte sedimentation rates following untreated acute respiratory

* By invitation.

illnesses. Throat cultures and serum specimens were obtained in the first week of renal disease and at 3-6 months and 12 months later. Antistreptolysin O (ASO), antihyaluronidase (AH), and antistreptokinase (ASK) antibody tests, as well as filter-paper electrophoretic patterns, were carried out on all sera.

Group A $\beta$-hemolytic streptococci were isolated on culture from 14 patients and from family contacts of 2 other patients. Organisms isolated included seven Type 12 strains, four Type 49 (Red Lake) strains, four nontypable strains, and one organism which failed to grow on subculture. Elevations of ASO titers were demonstrated during the acute renal disease in 24 patients. Prolonged elevations of ASO were observed in 50% of these patients. AH and ASK antibodies were not consistently elevated. The one patient, without demonstrable antibodies, had a Type 12 Streptococcus isolated. Serum albumin was consistently decreased during the acute illness in inverse proportion to the degree of albuminuria and edema. $\alpha_2$-Globulin was elevated and related inversely to the serum albumin levels. Both fractions returned to normal with clinical improvement. Gamma globulin levels were elevated in 10 patients during the acute disease and in 4 sera three to six months later. A significant correlation between $\gamma$-globulin levels and ASO titers was apparent (at the 5% level.) $\beta$-Globulin and $\alpha_1$-fractions were not consistently affected.

The data suggest that serum albumin and $\alpha_2$-globulin changes, associated with acute poststreptococcal glomerulonephritis, are related to the clinical severity of the disease. The changes in $\gamma$-globulin, however, appear to be specifically related to the immunologic response of the host to the previous streptococcal infection. Since the $\gamma$-globulin elevations reflect antigenic stimulation, this may be the explanation for the elevated levels of this protein fraction previously reported in this disease.

### 13. A New Abnormality of Tryptophan Metabolism in Phenylketonuria

SAMUEL P. BESSMAN and KEIYA TADA,*
University of Maryland

Studies of the aromatic amine excretion in phenylketonuria will be discussed. Two nitrogenous substances are excreted in excess in phenylketonuria, and one of these has been identified as indican. The excretion of indican has been found to be two to three times normal in this disease. Its excretion is directly related to the blood phenylalanine level and reverts to normal with the low-phenylalanine diet. This indicates that this is not a specific enzyme deficiency but that this wastage of tryptophan is caused by the phenylalanine abnormality. Even though the indican excretion is high in phenylketonuria, the rate of conversion of indole to indican is diminished in this disease. Tolerance curves for tryptophan and of tryptophan as indole and indican. The mechaindole will be presented which demonstrate the diminished utilization of tryptophan and wastage nism of this lesion and its possible relation to mental deficiency will be discussed.

* By invitation.

#### Discussion

DR. DONOUGH O'BRIEN, Denver: I wonder if you have worked out the absolute excretion coefficients for indican in these children; they would provide more convincing expressions of the data than the creatinine ratios.

DR. HENRY W. BAIRD III, Philadelphia: Dr. Baldridge, Dr. Borofsky, and I have given 5-hydroxytryptophan to children with phenylketonuria and have chemical data which are similar to those of Dr. Pare. We think that we have seen some clinical improvement in the children. (Baldridge, R.; Borofsky, L.; Baird, H. W.; Reichle, F., and Bullock, D.: *Proc. Soc. Exper. Biol. & Med.* 100:529-531, 1959). I wonder if Dr. Bessman would comment on this in view of his findings.

DR. HARRY A. WAISMAN, Madison, Wis.: Dr. Bessman's interesting presentation indicates that some additional information might be obtained by working with rats and it may well be that Dr. Bessman would find that the rat is a better animal for these studies than the child because what he is describing may very well be a disturbance in competitive enzyme systems. The hydroxylase that has for its substrate phenylalanine or tryptophane may be acting in such a manner that one or the other amino acid is sought for. In other words, the amino acids are competing for the same hydroxylase. This is similar to the situation that exists for the decarboxylase enzyme that removes $CO_2$ from phenylalanine and tryptophane. Apparently part of the mechanism that forms indican may be dependent upon the decarboxylase enzyme and perhaps also on the hydroxylase enzyme. Our studies in rats have shown that tryptophane is closely interwoven with phenylalanine metabolism. For example, the administration of tryptophane increases the phenylalanine hydroxylase content of the rat liver. The finding of indican is another clue that "imbalances" in enzyme activity may also exist to explain metabolic by-products. The finding by Dr. Bessman is a significant step forward in the understanding of the inter-relationships of all the aromatic amino acids in the disease phenylketonuria, whereas it was previously believed that only phenylalanine was involved.

DR. BESSMAN: Dr. O'Brien, we are trying to work out these excretion coefficients. We find that in general the total excretion parallels the creatinine coefficient. We need the creatinine coefficient because we were not sure of complete collection in a number of patients.

In answer to Dr. Berendes' first question, the tryptophane intake on our low-phenylalanine diet, is not one-third of normal as far as we were able to calculate, but, be that as it may, we now have a patient on the low-phenylalanine diet with phenylalanine added. This keeps the tryptophane constant. The same phenomenon occurs.

In regard to the xanthurenic acid determination, it's a method of Price. The serotonin in blood is a little lower but variable and sometimes the same as normal. On the low-phenylalanine diet it does in general increase, and it is in general lower in a patient with phenylketonuria.

The percentage diminution in serotonin and 5-hydroxyindolacetic acid is approximately the same as the percentage diminution we see in all the metabolites—from about 20% to 40%.

Now, we do not believe that this amount of alteration in blood serotonin reflects very much. It may indicate that there is a 30% diminution in hydroxylation of tryptophane. We oursèlves have published a paper on the effects of 5-hydroxytryptophane on hepatic coma and the electroencephalographic alterations that we observed. We do believe there can be symptoms resulting from deficiency of serotonin in the brain resulting from the inability of the liver to convert tryptophane to 5-hydroxytryptophane. This is a very exciting idea about the mechanism of coma. However, we don't believe that the patient who has phenylketonuria behaves like a patient who has hepatic coma. The EEG effects of 5-hydroxytryptophane are the same kind as the EEG effects that you described.

Dr. Baird, the question about the clinical experiment on the treatment of children with 5-hydroxytryptophane I think falls in the same answer. I don't believe that the only thing wrong with these patients is the deficiency of 5-hydroxytryptophane resulting in an intracerebral deficiency of serotonin.

In regard to this point, the work of Udenfriend recently showed that tryptamine is markedly increased in the brain on the administration of iproniazid. Apparently tryptamine is one of that galaxy of pharmacologically and probably physiologically active agents in the hypothalamus. We are interested in measuring these things and we believe that the tryptamine formation in patients with phenylketonuria may be even higher than normal. I would not be surprised to hear that if you measure indolacetic acid in the urine, that it is markedly increased in patients who have been given excess tryptophane.

In regard to Dr. Waisman's question, I wish we had some rats which had phenylketonuria. There seems to be a partial block in the metabolism of the indole nucleus in phenylketonuria. I do believe that Dr. Waisman is going to produce from his group some very important information which will lead to the elucidation of these lesions.

I do not believe that the absence of a single hydroxylase from a competitive basis is the reason for the indole lesion, because the first step in tryptophane utilization, that is, tryptophane peroxidase, is probably abnormal. There is competition at all levels in this lesion, and there is even competition in the handling of the indole itself.

### 14. Pathogenesis of Jejunoileal Atresia

W. A. BLANC, T. V. SANTULLI,* and D. H. ANDERSEN, Columbia

In 14 consecutive cases of jejunal or ileal atresia examined at the time of operation since 1954, squames or bile were present in material taken from the distal lumen. The distal segment showed chronic granulomatous lesions which could not be interpreted as resulting from ulceration and perforation of the proximal segment. Such findings would not be expected if atresia resulted from lack of recanalization of the embryonal intestine at the end of the second month (epithelial plug stage). Furthermore, the latter concept is debatable as far as jejunum and ileum are concerned.

Surgical and autopsy specimens of 37 cases of atresia were reviewed in an attempt to answer the following questions: (*a*) How common is "secondary" atresia and, (*b*) what are its causes?

(*a*) Material from distal or intermediate segments was available in 33 cases. These included cases of "membranous" occlusion which could conceivably result from lack of recanalization. In the majority of cases, there was microscopic evidence of scarring or of vernix granulomata, frequently with focal muscular defects. Swallowed squames, bile or both, were found distal to the obstruction in all 33 cases, indicating that occlusion occurred only in the fourth month or later.

(*b*) In the single atresias (11 cases), there were 1 case of malrotation, 1 case of cystic fibrosis of the pancreas (CFP), and 9 unexplained cases (3 with mucus inspissation [MuI] in intestinal glands).

In three double atresias with an intermediate loop, there were two unexplained cases and one with a ruptured omphalocele (all with MuI in the intestine, two with pancreatic concretrations without fibrosis or inflammation).

* By invitation.

In multiple atresias (nine cases), there were two malrotations and seven unexplained cases (three with minimal MuI in the intestine).

In 13 examples of double atresia with a large intermediate segment (calcified pseudocyst or matted loops with volvulus and calcified meconium peritonitis; Andersen, Zuelzer), there were five cases of CFP (one with malrotation), three with malrotation (one omphalocele), and five unexplained cases (two with extensive intestinal and pancreatic MuI).

The existence of "primary" atresia of the jejunoileum is questioned. The pathogenesis is still not clear in two-thirds of the cases, and the etiologic significance of MuI (? early CFP, or result of dehydration) found in half of the latter cases is unknown.

The various types of atresia and probably of stenosis appear to result from fetal mechanical injuries. The commonest is intrauterine volvulus, which may result from meconium ileus, malrotation, mesenteric anomalies, or persistent fetal umbilical hernia (Nixon).

### 15. A New Etiological Concept in Athyrotic Cretinism

R. M. Blizzard,* R. W. Chandler,* B. H. Landing, J. M. Sutherland,* V. M. Esselborn,* M. Pettit,* and G. M. Guest, Ohio State and Cincinnati

The role of thyroglobulin autoimmunization has recently been recognized as a contributing factor in the pathologic physiology of many thyroid diseases. Speculation as to the causal relationship of maternal thyroidal autoimmunization and athyrotic cretinism has been intriguing. Data obtained investigating this possibility are used as the basis of this report.

Antibodies to thyroglobulin were determined by RBC agglutination, complement fixation, agar-gel precipitation, and fluorescein-labeled-antibody staining method. The sera of over 30 cretins and their mothers and the sera of over 1,600 pregnant, or recently pregnant, women were tested for the presence of antibodies. In the latter group, when antibodies were found, the mothers and infants were evaluated for thyroid disease and their sera was titered for antibodies. Breast milk was also investigated for antibodies. Similar studies were also done at the time of delivery on immunized female rabbits and their offspring. Fetal growth and thyroid histology was evaluated in the latter.

Antibodies to thyroglobulin have been demonstrated in (*a*) both the sera of three mothers and their cretinous offspring (1 day to 2 weeks of age), (*b*) the sera of two mothers but not in their cretinous offspring (3 and 9 years of age), (*c*) the sera of two cretins (5 and 6 years of age) but not in their mothers' sera, and (*d*) the sera of six mothers bearing normal infants.

These data suggest that (1) placental transfer of maternal antibodies to thyroglobulin produces subsequent destruction of the fetal thyroid in some instances, and is an etiology of athyrotic cretinism (Group A), (2) the presence of such antibodies in the maternal circulation does not always produce cretinism (Group D), (3) all cretinism is probably not of this etiology, and (4) some cretins (Group C) may initiate their own autoimmunization process postnatally or even conceivably in utero.

The immunological concepts supported by data obtained from both human and animal subjects will be discussed.

### 16. The Mechanism of Aminonucleoside Nephrosis in Rats

Joseph D. Boggs * and Joel R. Stern,* Children's Memorial Hospital, Chicago

Introduced by David Yi-Yung Hsia

The injection into rats of an aminonucleoside (DARP) derived from Puromycin results in a nephrotic syndrome resembling that seen in humans. Studies were conducted to determine the mechanism of this disease in rats—whether the substance acted as an antimetabolite, participated in an antigen-antibody response, or was directly toxic to the kidney. DARP was introduced into young rats after a period of limited diet. Diet was so controlled that no weight gain was permitted over several days before introduction of the chemical. Control groups were permitted to feed ad libitum. In the restricted series, proteinuria was minimal; proteinuria was severe and prolonged in the control series. Farr and Smadel (1937) reported that the severity of nephritis produced by nephrotoxic serum introduced into rats was directly proportional to the level of protein in diets fed after injection. Alteration of protein after injection of DARP into the experimental group did not alter the results. High- or low-protein diets before DARP injection also failed to alter the course of the changes in the experimental group. Evaluations were based on urinary protein, serum cholesterol, and electrophoretic studies.

Additional studies were undertaken by introducing into the group of experimental animals various combinations of rats in parabiosis. Results suggest that DARP acts as an antimetabolite, interfering with an enzyme system as yet unrecognized.

* By invitation.

### 17. The Cytological Action of Human Serum on Certain Atypical Mammalian Cell Strains Propagated in Vitro

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Western Reserve and
Babies and Children's Hospital, Cleveland
Introduced by Samuel Spector

Normal human serum was found to have a marked cytotoxic effect on six atypical mammalian cell strains derived from malignant tumor or spontaneously transformed cell cultures. No such serum effect could be shown on three lines of relatively normal human cells and two such lines of rabbit origin. All of these cells were propagated in vitro in a synthetic medium containing 20% normal horse serum. The comparative cytotoxicity of the sera of 34 normal adults, 26 newborn infants, and 27 cancer patients was studied on strain U12, a typical transformed human fibroblast. This cell had undergone transformation in vitro, and so morphologically and physiologically it closely resembled a malignant cell. No difference could be demonstrated between the cytotoxicity of normal adult serum and cancer serum, but there was a significant reduction in the toxicity of neonatal serum. The toxicity of serum was manifested as virtually complete cell death within one hour's incubation at 37 C. With many of the cell strains, these effects were demonstrable in serum dilutions over 1:64.

Since the cytotoxicity was nondialyzable and could be inactivated by heating serum at temperatures greater than 45 C, the reaction was studied from the viewpoint of the complement-properdin system. Utilizing strain U12 as the model cell system, it was found that the reaction did not require the presence of properdin or intact complement and yet appeared to require the presence of substances resembling in their properties C'2, C'3 and C'4. Serum treated with edathamil trisodium did not affect the reaction, indicating that $Ca^{++}$ and $Mg^{++}$ ions were not required. Adsorption of serum with large number of cells eliminated the cytotoxicity and was associated mainly with an inactivation of C'2 and C'4. Horse, rat, rabbit, and guinea pig sera did not show any toxic activity. The addition of guinea pig serum to heat-inactivated human serum did not reconstitute the toxicity. The effects of lipid extraction suggest that the activity may be associated with the serum lipoproteins. It is possible that these observations reflect the presence of humoral mechanisms involved in a natural host resistance to cancer.

### 18. The Use of Methdilazine Hydrochloride in Electroencephalography of Children

L. G. BOROFSKY,* Temple
Introduced by H. W. Baird

Methdilazine hydrochloride is a derivative of phenothiazine and is closely related in structure to histamine. Preliminary animal investigation has revealed that the chief effect of nonlethal dose levels in experimental animals is sedation. Rapid absorption and total tissue clearance in 24 hours has also been noted in mice. Unpublished observations suggest that methdilazine is a potent antihistaminic agent in human subjects.

Methdilazine was used as routine sedation prior to electroencephalography in 25 children ranging in age from 2 months to 9 years. The clinical diagnoses on these patients included convulsive disorders, developmental retardation, cerebral palsy, rheumatic heart disease, and various endocrine disorders.

The oral dose was arbitrarily established at 1 mg. per kilogram of body weight for patients weighing less than 20 kg.; those weighing more than 20 kg. were given 20 mg. The drug was supplied in liquid form, containing 2 mg/5 cc., and in 4 and 8 mg. capsules. Electroencephalograms were obtained immediately after the drug administration and every 5 minutes thereafter for approximately 75 minutes.

Twenty-four of the 25 patients were clinically sedated. Sleep was noted within 35 to 55 minutes following drug ingestion in 22 patients. All of these children were easily aroused, although they were drowsy at the end of the observation period. Two children were quiet and drowsy but did not fall asleep. One child showed no sedative effect when given 1 mg. per kilogram of body weight of Methdilazine but was asleep and clinically sedated 50 minutes after receiving 2 mg. per kilogram of body weight on the following day.

There was no difficulty in administration of the drug. It seemed very acceptable in regard to its taste. One infant vomited shortly after drug ingestion but retained the drug without further difficulty on the following day. No side-effects other than drowsiness and decreased pupillary size were noted.

The initial portions of all tracings were obscured by movement artifacts. About 10 minutes before the child fell asleep, these disappeared in virtually all of the tracings. There was a minimal increase in $\beta$-activity in 20 of the 25 records at the time that sleep was first noted clinically. However, the records themselves appeared to be cleaner and were easier to interpret.

Electroencephalograms without sedation were obtained in nine of these children. There was no difference in the final interpretation of these records as compared to those following the administration of Methdilazine. The only difference was that those records obtained following the use of this agent were more easily readable.

Because the greatest incidence of EEG abnormalities appear during light sleep, an agent which would persistently produce light sleep has many theoretical advantages. Methdilazine appears to

* By invitation.

have this effect. In addition, it is a mild sedative which does not markedly alter cortical activity. It is effective within an hour of its administration and is readily acceptable. No untoward side-effects have been noted in the children thus far. On the basis of these results, it appears that Methdilazine is an effective means of reducing movement artifacts in the electroencephalograms of uncooperative children and of obtaining records during light sleep. Abnormal discharges have been distinctly preserved.

### 19. Patterns of Distribution of Multiple Facial and Pharyngeal Anomalies

JAMES F. BOSMA and SAMUEL G. FLETCHER,*
University of Utah

Anomalies in the region of the face and pharynx occur in a wide range of anatomical dispersion, extending from the cervical vertebrae to the lips and from the basicranium to the abdominal rectus muscles. This regional distribution of anomalies is identified in gradations within a series of 30 pediatric subjects evaluated in a continuing study of development of form and function in this region.

These subjects demonstrate various combinations of hypoplasia of cervical vertebrae, pharynx wall, palate, tongue, pterygoid apparatus, hyoid suspensory musculature, and musculature of the ventral body wall. Distinction of these hypoplasias may be made difficult by displacements of the mobile elements of facial skeleton—the mandible and the hyoid—and the general performance incapacity of this region which are associated with their manifestation. Their evaluation is facilitated by specialized techniques of visualization and roentgenography. Special methods of demonstration of motor performance have also been devised by which the associated respiratory obstruction, the deficiency of cough or of clearing of secretions from the eustachian apparatus and epipharynx, and the dysphagia are better understood. On the basis of these studies it has also been possible to anticipate further clinical problems and to devise continuing motor or orthodontic therapy.

This regional perspective of anatomical anomalies facilitates distinction of "primary" abnormalities of these structures from their distortions by impairments of the central nervous system, as in cerebral palsy or by myopathies.

### 20. Placental Insufficiency and Growth

MARY BOWEN * and RAY HEPNER,
University of Missouri

Aberrant growth in height and weight during the first six months of extrauterine life is frequently observed. Disease or malnutrition in the mother, prematurity, infections and malnutrition in early infancy, and emotional, environmental and socioeconomic factors have been implicated. More recently pediatricians and obstetricians have focused attention on the placenta as an organ of transport and have suggested "premature senility," "aging," and "placental insufficiency" as concepts for consideration. These latter investigators emphasized some related obstetric factors, smaller size of the neonate, and increased stillbirth and neonatal death rates in pregnancies characterized by abnormal or insufficient placentae.

The placental abnormalities enumerated are calcification, infarction, fibrinoid degeneration, thromboendarteritis, and syncytial budding with cytoplasmic concretions. These abnormalities are found in apparently normal pregnancies but are said to be commoner and severer in pregnancies complicated by hypertension, albuminuria, renal disease, or diabetes. Eastman reported that 63% of all placentae have evidence of the changes above. The cause of these changes is unknown. Reduced placental vascular function has been demonstrated in the same kinds of abnormal pregnancies in which placental structural changes are found.

Obstetricians have noted that the infant born of the toxemic mother does well—if it survives the immediate newborn period. Pediatricians have observed that physical measurements do not correlate directly with the infant's physiologic maturity.

In the present study, all placentae were examined for gross evidences of malformation or degeneration. Our observations may be less consistent or perhaps more crude than Eastman's in that our incidence of gross lesions of the placenta is 44%. Many severely affected placentae were delivered of women whose prenatal course was normal.

Growth curves of infants born with abnormal placentae are plotted on charts constructed from the data of Stuart and Meredith. An unselected group born in the same hospital during the same period is plotted similarly as a control. The infants born with abnormal placentae graduate from the 25th percentile in weight at birth to the 75th percentile by the third to the fifth month and from the 25th to the 75th percentile in height by the sixth month and maintain these positions to the first birthday. A small number of infants, premature birth by measurements but physiologically mature by examination and neonatal adjustment, had severe placental abnormalities, such as velamentous insertion of the cord, extreme thinness of the placenta, advanced circumvallate state, or universal fibrosis. These infants have little evidence of recovery from small physical size and remain apparently permanently stunted physically, although none have signs of retardation mentally, socially, or neuromuscularly. The control patients

* By invitation.

remain remarkably within the percentile distributions in which they were born.

Placental insufficiency may affect growth of the fetus in utero. Aberrant growth in height and weight in the first six months may be a reflection of intrauterine nutrition.

### 21. The Protective Action of Albumin in Bilirubin Toxicity in Newborn Puppies

WILLET R. BOWEN,* EVA PORTER,* and WILLIAM J. WATERS,
State University of New York, Syracuse

A bilirubin solution (100 mg. per kilogram, containing 40 mg. of commercial bilirubin [Fisher], 0.3 cc. of 0.75 N NaOH, 10 cc. of 0.05 M KH $PO_4$ at pH 7.6; solution neutralized to pH 7.8 with 0.05 cc. of 0.5 N HCl) was administered to twelve 1- to 7-day-old anesthetized (15 mg. of pentobarbital [Nembutal] per kilogram, intraperitoneally) puppies via a constant femoral vein infusion over a two-hour period. This was found to be a lethal dose to all puppies within three to six hours of the start of the infusion, with the production of marked jaundice, high serum bilirubin levels, and generalized staining of the brain. The tissues of these animals were analyzed immediately after death for their bilirubin content on a wet weight basis. The average concentrations are as follows: brain, 0.5 mg. %; muscle, 1.2 mg. %; fat, 3.0 mg. %; liver, 6.4 mg. %; spleen, 4.8 mg. %; serum, total, 36.5 mg. %, and direct, 4.8 mg. %.

The same solution minus the bilirubin was tolerated by 10 controls with no immediate ill effects.

Another group, of 13 1- to 7-day-old puppies, was given the same bilirubin solution but simultaneously received intravenously 1 gm. of a sodium chloride-free human albumin per 21 mg. of bilirubin administered. Ten of this group survived 24-48 or more hours, with restoration to normal activity in 12-24 hours. In addition, the jaundice was cleared and essentially no bilirubin was found in the serum, tissues, or brain, as contrasted with the first group.

Ten control puppies given the buffer solution plus albumin survived the 24- to 48-hour period with no apparent ill effects.

It would appear from the above data that albumin has a protective action in bilirubin toxicity of newborn puppies.

### 22. The Heart Sounds in the Newly Born Infant

M. BRAUDO* and R. D. ROWE, Toronto

A knowledge of the findings of auscultation of the heart of the normal newly born infant is necessary for the proper evaluation of the physical signs found in babies with respiratory distress, in whom congenital heart disease is always a diagnostic possibility.

The heart sounds and murmurs in the first week of life were studied in 80 newly born, mature infants whose delivery was uncomplicated and who were in no distress at birth.

Except for its loudness immediately after birth, the first heart sound is not remarkable.

The second heart sound is loud and single at birth. Slight splitting is detectable in more than 50% of babies after the age of 4 hours and in all babies over the age of 48 hours. The degree of splitting of the second heart sound is inversely proportional to the degree of T-wave positivity in lead $V_1$ in the electrocardiogram, and it is suggested that a rough assessment of the pulmonary artery pressure can be derived from a study of the second heart sound.

An early systolic ejection click is audible in all babies under the age of 2 hours. The incidence of this click diminishes rapidly in the first 16 hours of life but is still audible in 25% to 30% of babies throughout the first week of life.

Murmurs of various types were audible in 60% of babies. Ejection systolic murmurs were the commonest, and were heard in 57.5% of cases; continuous and crescendo murmurs were heard in 14% of babies; "pansystolic" murmurs were detected in 4% of babies.

The origin of these murmurs is discussed in the light of our knowledge of the circulatory hemodynamics in the early neonatal period. An attempt is made to explain why murmurs of ductal origin are heard relatively infrequently in the nursery.

### 23. Intra-Aortic Transfusion and Endotracheal Insufflation in the Resuscitation of Severely Anoxic Newborn Lambs

IRA K. BRANDT,* PETER CUNNINGHAM,* and HERBERT S. HARNED JR.,* Yale
Introduced by Nelson K. Ordway

Newborn infants who are unable to initiate breathing rapidly develop circulatory insufficiency which to some extent impairs their ability to respond to adequate oxygenation brought about by endotracheal insufflation. In addition, it is thought that an adequate pulmonary vascular tension may be a requirement for the initial lung expansion. The anoxic state has been treated with intra-arterial and intravenous injections of oxygenated blood with some success in restoring both circulation and oxygenation.

The present report describes some experiments in which the effectiveness of intra-aortic transfusion and endotracheal insufflation were studied during the attempted resuscitation of severely anoxic newborn lambs.

* By invitation.

The animals were delivered by hysterotomy; their respiratory passages were occluded, and their umbilical cords were ligated after catheters had been inserted into the aorta through each umbilical artery. The latter permitted continuous recording of the aortic blood pressure and periodic sampling of blood. Continuous electrocardiographic recordings were also made.

Within a few minutes of the induction of asphyxia there was a rise in the plasma lactate and potassium concentrations and carbon dioxide tension and a fall in the pH and in the concentration of bicarbonate and of pyruvate. These chemical alterations were accompanied by a precipitous fall in the heart rate due to heart block, and this was shortly followed by a fall in the systolic and pulse pressures. The heart block disappeared within a few minutes, but other electrocardiographic changes compatible with anoxia were noted and the cardiac output remained low.

After 5 to 12 minutes of asphyxia rapid intra-aortic transfusion of heparinized arterial blood taken from the corresponding ewe was performed. This produced only a transient rise in blood pressure and ventricular rate, which in a few minutes was no longer evident. The severely depressed clinical condition of the lambs was not altered by this procedure.

Endotracheal insufflation was then performed with use of oxygen at a pressure of about 35 cm. of water. This produced a striking and sustained improvement in systolic pressure and ventricular rate and in the electrocardiographic pattern. In addition, the progressive increase in lactacidemia was arrested and the acidosis and hypercapnia were somewhat ameliorated. The pyruvate concentration rose to its preasphyctic level, and the potassium fell to its previous level or below. The condition of the animals improved dramatically; within minutes return of reflexes and spontaneous movements were noted, and in a short time they were able to carry on respiration unaided. However, none revived completely and eventually all animals died.

A second intra-aortic transfusion given to some animals produced an additional increase in the systolic pressure, which was sustained in contrast to the evanescent effect of the first transfusion.

### 24. The Determination of the Electrical Conductance of Microsamples of Sweat as a Means of Diagnosis of Congenital Fibrocystic Disease

HENRY B. BRUYN, University of California

The determination of the sodium and chloride content of the sweat has become the principal laboratory tool in establishing the diagnosis of congenital fibrocystic disease. Samples of sweat in volumes from 0.2 to 2.0 ml. are analyzed quantitatively after collection on guaze pads or plastic bags. The collection period is not without discomfort to the patient, and one case of "heat shock" and death has been recorded.

The electrical conductance of sweat was studied by Lundegren, in 1955, who showed that very small samples could be tested and that there was a high correlation between the sodium chloride content and the electrical conductivity. Schwachman, in 1957, using larger samples of sweat, demonstrated a very close correlation between electrical conductivity and sodium chloride content.

The present report concerns the use of a compact new instrument used at the bedside and designed to measure the conductance of samples in a volume of 2λ. Platinum electrodes are mounted in a plastic collection piece which is drawn across an area of skin damp with sweat. The reading of conductance is obtained immediately. With use of this instrument on standard sodium chloride solutions, a greater accuracy and consistency is found than with the flame photometer. Correlation with the quantitative sodium and chloride determinations on sweat specimens is consistently close. The determination of the conductance of saliva has been studied on a small scale and demonstrates the extreme variability of saliva specimens in their electrical conductance within short periods of time.

With this instrument, a diagnostic test may be performed rapidly after a brief period of sweat induction on an extremity and carried out by encasement in plastic and the application of local heat for 20 to 30 minutes. While the conductance in micromhos can be translated to millequivalents of sodium chloride and the correlation with quantitative determination is close enough for clinical purposes, it is proposed that the conductance itself be used as a diagnostic criteria. Levels of conductance found in fibrocystic children are clearly distinguished from those of normal children.

### 25. Patterns of Electrolyte Balance in the Premature Newborn Infant

JOSEPH BUTTERFIELD,* University of Colorado
Introduced by Lula O. Lubchenco

The ability of the small premature infant to adapt to an early extrauterine environment may, in part, be related to the management of body water and electrolytes. It was the intent of this project to study patterns of electrolyte balance in the normal premature newborn and to extend these attentions to the variations from normal that might be seen during illness.

Twenty-four premature infants were observed during the first three weeks of life while being maintained on an electrolyte balance regimen. With

* By invitation.

three exceptions, birth weights were less than 1,600 gm. Five per cent glucose in water was offered at about 24 hours and followed in 12-24 hours by a partially skimmed milk and carbohydrate mixture (Dalactum); each batch of which was analyzed for sodium, potassium, and nitrogen by this laboratory. Sodium, potassium, and nitrogen determinations were done on daily collections of urine and stool. Serum electrolytes were measured on four of the first seven days and at weekly intervals thereafter.

The infants were divided into three groups according to their clinical histories and the time interval studied.

1. Six infants were studied during the first week of life at a time when they were free of disease. During this time they all had a consistent loss of sodium, ranging in cumulative deficit from 5-13 mEq. per kilogram. Serum sodium values for the group varied from 127-148 mEq. per liter, with fluctuations of individual infants ranging from 2-16 mEq. Potassium balance became positive soon after milk feedings were begun. Serum potassium values for the group ranged from 4.0-7.8 mEq. per liter, with a mean peak value of 6.8 mEq. Nitrogen balance also became positive when feedings were started but occurred later than the onset of potassium retention.

2. Thirteen infants were studied during the first week of life at a time when they were ill with hyaline membrane disease, sepsis, sclerema, or some undiagnosed illness. Some of these infants demonstrated balance patterns very similar to those in Group 1, while others showed marked variation in their electrolyte and nitrogen balance curves. This group had serum sodium values that ranged from 103-150 mEq. per liter, with fluctuations of individual infants ranging from 1-43 mEq. Of the seven patients with serum sodium values of 135 or less during the first week of life, six were in this group. Serum potassium values ranged from 4.5-10.4 mEq. per liter, with a mean peak value of 7.5 mEq.

3. Twelve infants, six of whom were ill, were studied during the second and third weeks of life. All of them showed a leveling off with the trend toward positive cumulative sodium balance and a continued retention of potassium and nitrogen.

It was noted that apparently healthy premature infants had a characteristic pattern of electrolyte and nitrogen balance. Sodium loss occurred in the first week of life, followed in the second and third weeks by a shift to a positive balance. Potassium retention began soon after feedings were initiated and was soon followed by conservation of nitrogen. During illness, variations from the usual excretion pattern may be considerable and vacillation in serum electrolyte values may be marked.



## 26. A Comparison of Trimethadione and Its Demethylated Product in the Control of Petit Mal Epilepsy

HARRIE R. CHAMBERLIN,* THOMAS C. BUTLER,* and WILLIAM J. WADDELL,* University of North Carolina

Introduced by Edward C. Curnen

Several well-recognized anticonvulsant drugs (mephobarbital, metharbital, mesantoin, trimethadione, and paramethadione) are demethylated by the liver. Their demethylated products accumulate for several weeks if daily dosage is maintained. The demethylated products of the last two compounds have not previously been tested for their antiepileptic effects. The present study was designed to determine the effectiveness in petit mal epilepsy of 5,5-dimethyl-2,4-oxazolidinedione (DMO), the demethylated product of trimethadione (Tridione).

DMO, prepared in our laboratories, was administered to four children with classical petit mal seizures (characterized by a three-per-second spike-and-wave EEG pattern) in doses ranging up to 76.5 mg. per kilogram per day for periods of 70 to 159 days. DMO plasma levels have been followed (by the use of an ultraviolet spectrophotometric method), and the number of observed petit mal seizures per day have been charted for periods ranging from 9 to 20 months. In three children treated with DMO seizures were completely controlled at plasma levels of 850, 1,020, and 600 mg. per liter. In all three seizures recurred within 18 to 35 days after discontinuance of the drug. Trimethadione was then administered to the first child in equimolar doses. Seizures were again controlled when the DMO plasma level had attained the approximate range which had previously been associated with control. The response in the second child was similar. The third child, however, still has occasional petit mal episodes on a dosage of trimethadione productive of a far higher plasma DMO level than that which earlier was associated with control.

The fourth child experienced a gradual reduction of her polymorphonuclear leukocyte count to 1,500 per cubic millimeter over a period of five months of DMO administration. Although the level of DMO in the plasma was increased to 1,040 mg. per liter, only a moderate reduction in the frequency of seizures was achieved. It was not deemed advisable to determine whether trimethadione would produce comparable results. No other toxic effects of DMO were noted, although one child experienced his only grand mal seizures at DMO plasma levels of approximately 400 and 100 mg. per liter.

The findings indicate that the administration of DMO produces effects similar to those in re-

* By invitation.

sponse to trimethadione. In addition, the results of the study reemphasize the extremely slow rate of elimination of DMO from plasma. The average rate of decline of the plasma concentration was 7% per day after discontinuing medication. As a consequence "plateauing" of the plasma level following the initiation of a fixed daily dose is attained slowly; calculations based upon the average rate of elimination indicate that a period of 30 days is required for the plasma level to reach 90% of that achieved at full equilibrium. It was also demonstrated that the control of seizures persists for many days after the DMO level in plasma has declined below that initially required to achieve control.

There was no indication that trimethadione, produced by the addition of a methyl group to DMO, is superior to DMO itself in the control of petit mal epilepsy.

### 27. Experimental Hyperparathyroidism: Its Effect on Magnesium and Electrolyte Metabolism

DONALD B. CHEEK and HSI C. TENG,*
University of Texas Southwestern

Hyperparathyroidism, though rare in children, gives rise to sudden weight loss, lethargy, muscle weakness, dehydration, irritability, and, on occasion, to convulsions. Investigations were undertaken on the young rat (80-100 gm.) to determine the nature of electrolyte changes in the muscle, bone, plasma, and urine, that occur when three injections (100 units per injection) of commercial parathyroid extract (P. T. E.) are given at about 12-hour intervals.

Some rats were allowed diet (Group 1), while others were given 10% glucose only (Group 2) during the entire 45-hour experimental period prior to killing.

Changes in magnesium metabolism were found. The plasma Mg concentration rose in rats receiving P. T. E. There was also a 20% increase in muscle Mg content ($P<0.01$) in rats receiving diet. A smaller increase was found in rats receiving glucose only (6%; $P=0.05$). There was a trend toward a low concentration of bone Mg.

The experimental rats (Group 2) lost 12% of their initial body weight (control lost 4%), and during the last 30 hours of the experiment there was a rise in urine Na, Cl, and P excretion. Plasma Na and Cl concentration in Group 1 rats fell, while bicarbonate content rose to 30 mEq. per liter. The plasma pH did not rise above 7.45. Plasma concentration of Ca rose to 14 mg. %. Muscle Mg, but not Ca, increased, and muscle K showed a slight but definite increase ($P=0.05$). On the other hand, there was a loss of muscle Cl and Na ($P<0.01$).

That bone resorption was taking place was evidenced by the 200% rise in renal calcium content. While bone Ca concentration fell with a suggestion of fall in bone Mg, there was no change in bone Na and $H_2O$ concentration.

These experiments suggest that the weight loss, dehydration, and lethargy of hyperparathyroidism may be due to the coincidental depletion of E. C. V. and of Cl and Na. The increase in muscle Mg and K was of interest, since other experiments on magnesium and potassium loading in "hyperparathyroid rats" (Selye: *Endocrinology* 63:216, 1958) demonstrated highly toxic effects of these ions. It may be that there is difficulty in excretion of magnesium ion if dietary loads are added to the magnesium liberated from bone. Under circumstances of high calcium excretion, as present here, some reduction of magnesium excretion can be expected. The entrance of excess magnesium ion into muscle could result.

### 28. Melituria of Premature Infants

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It is the purpose of this preliminary report to present data indicating an increased incidence of melituria in premature infants.

A group of 77 well premature infants has been studied to date. The gestation period ranged from 28 to 38 weeks, with birth weights from 1,000 to 2,438 gm. The infants were fed three different types of formulas: a lactose-containing, isocaloric formula; a lactose-free, isocaloric formula, and a lactose-containing formula of 40 Cal. per ounce; these are hereafter referred to as Formulas 1, 2, and 3, respectively. Of the total of 253 urines analyzed, 135 specimens were obtained from 43 infants receiving Formula 1. The remaining 118 urine specimens were obtained as follows: 90 specimens from 18 infants receiving Formula 2; 16 specimens from 4 infants receiving Formula 3; and 12 specimens from 12 newborns prior to the initiation of any milk feedings. All urine samples were examined by one or all of the following techniques: paper chromatography, qualitative Benedict's test, or the Somogyi-Nelson method. Forty-one specimens of blood were obtained from 12 infants at 1, 2, 3, 4, 24, 48, and 96 hours of age and analyzed by paper chromatography and the Somogyi-Nelson techniques for sugar.

These studies reveal that 88 of the 135 urines (66%) obtained from infants receiving Formula 1 had a positive Benedict's reaction and 129 of the 135 urines (96%) had reducing substances in their urine by the chromatography technique, i. e., glucose, lactose, galactose, and occasionally arabinose either singly or in some combination. Six of twelve urine samples obtained from 12 infants before feedings were initiated had a positive Benedict's reaction. In 11 of the 12 samples, reducing

* By invitation.

substances were present by chromatography. Twenty-nine of ninety urine specimens (32%) obtained from 18 infants who received Formula 2 had a positive Benedict's reaction; the sugar was usually glucose by chromatography. With the use of Formula 3, fifteen of the sixteen urines specimens obtained from four infants had a positive Benedict's reaction and glucose, galactose, and lactose were observed by chromatography.

Quantitative determinations of total urinary sugar revealed great variation depending upon the type of feeding. While infants receiving Formula 1 had mean values for urinary sugar of 40 mg. %, those fed Formula 2 had values below 25 mg. % and those receiving Formula 3 had values above 100 mg. %.

The range of values for blood sugar in the first day of life was between 6 and 58 mg. %; from 24 to 96 hours, between 28 and 87 mg. %. Low blood sugar values were occasionally accompanied by high urinary values for sugar.

These data reveal that both the incidence and concentration of sugar in the urine of formula-fed premature infants is much greater than that noted in breast-fed infants by Haworth and MacDonald (*Arch. Dis. Childhood* 32:417, 1957).

## 29. The Relationship of Amino Acids to Idiopathic Hypoglycemia

W. A. COCHRANE * and F. MOYA,*
Dalhousie Medical School, Halifax
Introduced by Dr. R. Slater

In 1956, Cochrane et al. (Cochrane, W.; Payne, W.; Simpkiss, N., and Wolfe, L.: Familial Hypoglycaemia Precipated by Amino Acids, *J. Clin. Invest.* 35:411, 1956) described the fall in blood sugar following the administration of the amino acid L-leucine to three infants suffering from spontaneous hypoglycemia.

At the Toronto Hospital for Sick Children five cases of idiopathic infantile hypoglycemia were observed following the administration of L-leucine (0.15 gm. per kilogram) by mouth. All showed a marked fall in the blood sugar resulting in convulsions in four of the five cases. Two cases developed symptoms within 15 minutes of oral feeding. Electroencephalograms were done in two patients before the oral leucine feeds and were normal. Gross abnormality of the EEG developed 25 and 30 minutes after L-leucine, which gradually returned to normal following glucose administration by gavage.

Other amino acids, L-tryptophan and glycine, were administered without effect. D-Leucine similarly produced a marked fall in the blood sugar.

Treatment with cortisone only was compared in two cases to the postprandial feeding of extra carbohydrate to offset the hypoglycemia, which may be related to the L-leucine in the diet. Blood-sugar determinations done every two hours throughout the day revealed a particular "swinging pattern" which was not markedly altered by the use of cortisone or extra carbohydrate, although the blood-sugar values were increased.

Animal experiments included the study of blood-sugar changes following the intravenous administration of L-leucine to rabbits, dogs, and rats. In the latter species the blood sugar level was slightly decreased.

Two dogs were prepared by the insertion of cannulae into the portal and hepatic veins and administration of L-leucine into the portal vein. Simultaneous blood-sugar determinations at 10-minute intervals revealed a slight fall in the blood sugar from the hepatic veins but no change in the portal venous blood.

Preliminary in vitro studies using rat diaphragm indicate that in the presence of L-leucine the rate of disappearance of glucose from the incubating medium is increased as much as 30% to 50%.

In vitro experiments are being carried out to observe the effect of insulin and leucine and other amino acids on glucose uptake by rat diaphragm, liver slices, and epididymal fat pads.

Possible explanations of the effect of L-leucine on glucose metabolism would include (*a*) a central liver effect in blocking glucose release; (*b*) an intracellular chelating effect of L-leucine in binding zinc in the islets and freeing "soluble" insulin (this possibility is suggested by the work of Maske [Maske, H.: Interaction Between Insulin and Zinc in the Islets of Langerhans, *Diabetes* 6:335, 1957], who demonstrated the ability of certain substances, including amino acids, cystein and histidin, to bring insoluble zinc-insulin complexes into solution by chelation of zinc); (*c*) a peripheral effect different and unrelated to insulin action.

### Discussion

DR. ROBERT KLEIN, Pittsburgh: I think maybe I can help a little bit on ruling out some of the problems. I think that this actually is a very important contribution to a general understanding of some of these carbohydrate problems as well as to the specific syndrome.

I don't want to cite Dr. Mirsky's original animal work because I am not absolutely sure of the timing. I do know that in the human work with which I have had some contact, the effect of the tryptophane derivatives is very difficult to demonstrate in any individual without some diminution of insulin-secreting ability. In addition, as we reported a couple of years ago, with indolacetic acid we see no changes in normal humans. You can only see this effect in diabetics while they are still able to produce insulin. The effect does

* By invitation.

not take place in the times that have been studied here. It is a very slow effect over a five-hour period. So I would think that this is a different mechanism and the obvious problem now is to eliminate the question of whether it is insulin-sensitive or insulin-dependent, and I think that this will be an enormous help in our understanding of this problem.

DR. WILLIAM K. SCHUBERT, Cincinnati: I would like to add five cases to Dr. Cochrane's material and to support his suggestion that family members of patients with leucine-induced hypoglycemia be tested. In one of our families the index child had convulsions and was mentally retarded at the time of diagnosis, age 5 years. The mother gave a history of convulsions in childhood and demonstrated a typically positive leucine test with hypoglycemic symptoms at age 40. She is normal mentally and has no seizures at present. We have had good results with postprandial feedings and I would like to ask Dr. Cochrane to comment on therapy in these patients a bit more.

DR. HULDA WOHLTMANN, St. Louis: Dr. Cochrane, did you run any 24-hour blood-sugar curves on the children whom you studied, and, if so, did they demonstrate any marked variation in their blood-sugar levels from hour to hour? Dr. Hartmann Sr. and I have recently studied two children who were leucine-sensitive. One of these children was extremely sensitive. When only one-fourth the suggested test dose of L-leucine was used this infant's blood sugar would fall to near zero levels. When the blood sugar was tested to some of the intermediate products of leucine metabolism, the results suggested that mild hypoglycemia resulted. When these results were interpreted in view of the 24-hour blood-sugar curves, the results were equivocal; whereas the response to L-leucine always produced severe hypoglycemia.

DR. MELVIN GRUMBACH, New York: I'd like to congratulate Dr. Cochrane on this observation which has been of great help to all of us in investigating the waste basket of idiopathic hypoglycemia in infancy.

I'd like to describe briefly some observations of an infant that we recently made at Babies Hospital and to ask for some help from Dr. Cochrane in setting up criteria with regard to the significance of the fall in blood sugar in the infants who start out with low plasma levels.

This patient responded to L-leucine with an appreciable fall and with, on one occasion, symptoms of hypoglycemia. We also tested several other amino acids, and, with DL-isoleucine, with DL-valine and with L-valine among others, there seemed to be a tendency for the blood sugar to fall in the same period of time that we observed a fall with L-leucine.

The other question I would like to ask Dr. Cochrane concerns the difference in dosage of ACTH and of steroid required to control these children. It seems that appreciably more steroid than ACTH is required to control these infants.

DR. ROBERT A. ULSTROM, Minneapolis: As you know, we have followed your work with great interest and have confirmed your findings in a number of cases. We have also been hoping to get some lead on the mechanisms of this type of reaction to L-leucine and have investigated the steroid metabolism of a number of these children. We have no results that resemble clear-cut cause and effect relationships.

However, approximately half a dozen children that have had definite hypoglycemia do respond to L-leucine. We have quite consistently seen a fall in the blood steroid level during the period of administration of L-leucine. This is quite in contrast to what one could normally expect during the development of hypoglycemia. There is, however, a diurnal variation in the blood steroid level, and since we begin the test at 8:00 a.m., by 10 o'clock there would normally be a drop. We felt that evaluation by urinary steroids excretion might be of additional help. Although we found no low steroid output in these patients, we do find a reversal of the diurnal rhythm in fractional urine collections collected on the day of L-leucine administration.

I have no real hypothesis as to how these observations apply, nor do I have any ideas regarding the great effectiveness of ACTH in these children. I think Dr. Grumbach's observations on the difference in ACTH and steroid dosage level that these children require is similar to that Dr. McQuarrie made in his early work on the subject. ACTH was very effective in this disease and steroid was relatively ineffective unless very large doses were given.

I'd like to add a follow-up on a number of patients originally recorded 10 years ago by Dr. McQuarrie as idiopathic hypoglycemosis. We have been rather disappointed as we have evaluated the CNS picture of these children. Even though we felt that the blood sugars were fairly adequately controlled, mental retardation and seizures have developed.

DR. COCHRANE: First, I would like to thank the discussers for their remarks.

Dr. Klein, I am very pleased to hear your comments with regard to "insulinase inhibition." In Dr. Mirsky's studies the blood sugar changes following tryptophane administration to rats were evident after one hour. It would appear from his experiments and confirmed by your remarks that the "insulinase inhibition" effect is much slower than would be necessary to explain the "leucine hypoglycemia." The hypoglycemia fol-

lowing leucine may occur within 10 to 15 minutes in some children following the administration by mouth of the test substance. I personally feel that the effect of leucine is independent of insulin but as yet cannot support this impression by experimental evidence.

Dr. Schubert's remarks describing five further "leucine-sensitive" hypoglycemic cases is most interesting. The finding of hypoglycemia in a 40-year-old mother of an affected sibling given leucine is most interesting. We have induced hypoglycemia in two parents of hypoglycemic infants who gave a history of convulsions as an infant. We feel the parents should be tested with leucine and carefully observed.

The use of extra carbohydrate 20 to 30 minutes after meals along with ACTH is probably the treatment of choice at the present time. It would be worth while in severe hypoglycemic infants sensitive to leucine, to place them on a low-leucine diet to observe their response. A deficient intake of this essential amino acid would result in failure to grow, but if the hypoglycemic attacks were controlled and the danger of permanent mental retardation reduced this form of therapy would be justified.

Dr. Wohltmann has described hypoglycemic infants not responding to leucine, and we have classified these as "leucine-insensitive" cases. Dr. Wohltmann's careful control studies would indicate that only leucine was capable of inducing hypoglycemia.

In two patients we determined the blood sugar every two hours throughout the day while (*a*) on a normal diet, (*b*) while receiving cortisone, and (*c*) while receiving a normal diet and extra carbohydrate 30 minutes after feedings. There was a peculiar "swinging pattern" in the blood sugars with occasional low blood sugars recorded even while the patients were receiving steroids and extra carbohydrate. Therefore frequent blood sugar determination should be done while the patient is on steroid or hormonal therapy to observe any evidence of subclinical hypoglycemia.

Dr. Grumbach, we have carried out blood-sugar determinations every 30 minutes for 2 hours in some of our patients, and there is a gradual fall in the blood sugar, but not in comparison with the rapid fall in blood sugar following leucine administration. In the experiments using other amino acids and blood-sugar determinations made every 10 minutes, no similar fall in the blood sugar occurred comparable to the results following leucine administration.

Dr. Ulstrom, thank you for your comment, and since time is short perhaps I can talk to you privately.



### 30. Urinary Excretion of Corticosteroids in Infants: The Response to Surgery and Corticotropin (ACTH)

ELEANOR COLLE* and ROBERT A. ULSTROM, University of Minnesota

Serial 24-hour urine samples from 10 infants undergoing major surgery in the first month of life and four infants with hemolytic disease subjected to single or multiple injections of corticotropin (ACTH) have been studied. Porter-Silber chromagens have been measured before and after incubation with $\beta$-glucuronidase; free cortisol has been determined by a specific isotope dilution method and qualitative patterns have been further studied by paper chromatography. The quantitative data has been compared with similar samples collected from 51 normal newborn infants.

In five infants operated upon during the first four days of life, no rise in corticoid excretion was observed following surgery. One infant in this group was challenged with corticotropin on the sixth postoperative day. Following this, urinary excretion of corticosteriods was five times higher than that found following surgery at 3 days of age. In contrast, the excretion of corticoids was high on the first or second postoperative day in the five infants studied after surgery performed after the first week of life. Values gradually decreased thereafter. One infant in this group received corticotropin on the fifth postoperative day. His urinary excretion of steroids was then equal to but did not exceed his excretion on the day of his surgery when he was 8 days old. Increased corticoid excretion following single or multiple injections of corticotropin during the first week of life did not appear in four infants with hemolytic disease. One infant in this group, who had shown no increased steroid excretion after corticotropin on the fourth day of life, demonstrated a threefold increase in excretion as a response to the same corticotropin dosage on the seventh day of life.

The oldest infant (one month of age) had the lowest ratio of free:glucuronide conjugated steroids. No differences in this ratio between the other infants was, however, discernible.

Cortisol represented only a small percentage of the Porter-Silber chromagens in the free fraction in all infants. Tetrahydrocortisol and tetrahydrocortisone were identified as the major components of the free steroid fraction in the infants reported above.

In four infants, reextraction of the free compounds with ethyl acetate after complete extraction with methylene chloride resulted in additional recovery of steroids more polar than tetrahydrocortisol which react with phenylhydrazine. The major compound in this fraction has been tenta-

* By invitation.

tively identified as 6-$\beta$-hydrocortisol. In the younger infants, the additional steroids extractable only with ethyl acetate contributed up to 25% of the total Porter-Silber chromagens, while in the older infant they contributed less than 5% to the total. Although this represents an important pathway of cortisol metabolism in the newborn, consideration of the additional steroids extractable only with ethyl acetate does not eliminate the differences in steroid response to surgery and corticotropin between the younger and older infants. The studies emphasize the differences with age in the metabolic disposition of adrenal corticol steroids.

### 31. Role of PAP (Eaton-Liu) Virus in Childhood Respiratory Disease

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The indirect fluorescent-antibody technique was employed to determine if PAP virus or an agent antigenically related to it plays a role in childhood respiratory disease. Tests for antibody were performed by incubating serial dilutions of the patients' acute and convalescent serums on slides containing a thin section of chick embryo infected with PAP virus. The slides were then washed to remove serum components which did not react with the infected chick-embryo tissue section. The final step involved the addition of an antihuman-globulin serum (prepared in rabbits) conjugated with fluorescein which stained antibody reactive with PAP antigen when the infected section was viewed by ultraviolet microscopy.

One hundred ten infants and children with undiagnosed lower respiratory tract illness were studied for evidence of PAP infection. Of this group, 18 (16%) developed fourfold or greater rises in PAP antibody. Of 70 undiagnosed pneumonias, 14 (20%) developed an antibody rise for PAP. Two (10.5%) of nineteen patients with bronchiolitis and two (12.5%) of sixteen patients with pharyngitis and bronchitis developed an antibody rise. During the study period from October, 1957, through January, 1959, serologic evidence of infection was observed during 10 of the 16 months. No antibody rises were detected in a control group of 64 infants and children hospitalized during the same period who were free of respiratory disease during their period of observation. In addition, PAP antibody tests were performed on the sera of 33 children with lower respiratory tract illness from whom HA-1, HA-2, CA, Asian influenza A, or adenovirus was isolated or who developed serologic evidence of infection with one of these agents; no PAP antibody rise was detected in this group.

These findings suggest that PAP virus or an agent (or agents) antigenically related to it was associated with 16% of the undiagnosed lower respiratory illness in Washington during 1957-1959. Since 60% of cases of lower respiratory illness were etiologically undiagnosed, an estimate of the contribution of PAP virus to lower respiratory tract disease would be 10%.

#### Discussion

Dr. C. Henry Kempe, Denver: It has been stated that the PAP virus is inhibited by streptomycin, using the Coombs technique, and clinical studies suggest that the tetracyclines are able to affect the febrile course of primary atypical pneumonia. Have the authors attempted virus isolation in this study or was it limited to serologic approaches? Did the clinical material contain antibiotics for tissue culture inoculation and could these have masked virus?

Dr. Alfred L. Florman, Manhasset, L. I., N. Y.: This is a very nice example of how new tools help us to empty the big wastebasket of primary atypical pneumonia. It was not clear from the presentation whether all the individuals who were positive with the Eaton-Liu test, also had positive cold agglutinin and streptococcal MG reactions. Would you please elaborate?

Dr. Edward Curnen, Chapel Hill, N. C.: I am very much interested in the use of this refined technique for the study of a problem which has puzzled us for a number of years. As you know, several different agents were implicated as possible causes of this disease without clear indication as to which was responsible. There was evidence also that in different patients different agents had had an etiologic role. In some of the patients who had agglutinins for Streptococcus MG and cold agglutinins for human erythrocytes, another serologic reaction was also detected by Thomas et al.

(Thomas, L.; Curnen, E. C.; Mirick, G. S.; Ziegler, J. E., Jr., and Horsfall, F. L., Jr.: Complement Fixation with Dissimilar Antigens in Primary Atypical Pneumonia, *Proc. Soc. Exper. Biol. & Med.* 52:121-125, 1943.)

They showed that serum obtained from these patients during convalescence often gave a positive complement-fixation reaction with antigen prepared from a variety of different normal or infected tissues, especially mouse lung but also normal chick embryo yolk sac. Therefore I would like to inquire whether this test was carried out as well as that for cold hemagglutination and Streptococcus MG agglutination with serum from these patients. It would also be informative to know whether, in addition to tests for fluorescent antibody employing embryo tissue infected with Eaton agent, tests were

* By invitation.

also performed using tissues from normal embryos and from embryos infected with other agents.

DR. HEINZ F. EICHENWALD, New York: I'd like to ask if there was any clinically detectable difference between patients that did have a rise in antibodies and the patients that did not. Were there any differences in white count, degree of temperature elevation, duration of the disease, or anything that one might be able to describe clinically?

DR. ROBERT H. PARROTT, Washington, D. C.: Dr. Cook made me promise that I would try to answer some of the clinical questions. First, it should be made clear that the clinical picture in the 18 children reported was not uniformly what is described as primary atypical pneumonia as a clinical syndrome. Of the 18 Children's Hospital patients, 14 had bronchopneumonia clinically and by x-ray. Three of these clinically had bronchiolitic bronchopneumonia. Four children had bronchitis. One of these also showed spinal fluid pleocytosis and other signs of aseptic meningitis. There were sufficient central nervous system signs in two others that spinal taps were performed. There was no pleocytosis in these.

The fever in five of the patients was over 102 F and in eight was over 104 F. A majority of the patients were febrile for four or five days. The point is that if these studies are confirmed, then the primary atypical pneumonia virus is capable of causing more than primary atypical pneumonia as we have known it clinically.

It should be pointed out that the children who were studied were selected from those who were sufficiently ill to warrant hospitalization and thus were selected for relative severity of illness. We have no studies for PAP antibody in children with relatively minor respiratory tract illness.

The laboratory part of these studies was done in about a two-month period and the clinical materials have been collected over more than a year's period; the serums had not been tested for cold agglutinins and for Streptococcus MG antibody. Thus, we cannot answer the obvious question as to whether a rise in these antibodies accompanied a rise in antibody to primary atypical pneumonia virus.

We cannot say anything about the clinical effectiveness of the tetracyclines or chloramphenicol in these children since not enough patients are included and no attempt was made to control the therapy. The antibiotic used in most of these children was penicillin. Only five of them had received tetracycline or chloramphenicol therapy. The duration of illness in these patients was not notably different from that in those receiving penicillin.

DR. COOK: Dr. Kempe, I would like to stress that we made no actual virus isolations in this study. When the study was initiated, we did not contemplate studies with PAP pneumonia, and all of our throat washings and throat swabs were taken in media containing broad-spectrum antibiotics. At present we are collecting specimens that would be suitable for attempts at virus isolation.

We certainly do agree that there is evidence in the literature that streptomycin and tetracycline do have an effect against this organism although we have not done studies on this.

As Dr. Parrott has pointed out, agglutinins and Streptococcus MG patterns were not done in the childhood studies.

We have not tried complement-fixation tests with normal tissue antigen in the human group. In some of our laboratory studies with animals we have noticed that animals infected with this virus do indeed develop complement-fixing antibodies to normal antigens.

## 32. The Effect of Acetazolamide (Diamox) on Sickle-Cell Anemia

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Introduced by Henry K. Silver

Although some recent reports indicate that acetazolamide (Diamox) may be of value in the treatment of sickle-cell anemia, other investigators have not noted any changes in the clinical course of patients treated with this drug. The following studies were carried out to further elucidate the effect of acetazolamide on the course of the disease as demonstrated by changes in hemoglobin, hematocrit, reticulocyte count, and erythrocyte survival of patients with sickle-cell anemia.

Three adults and two infants with sickle-cell disease were studied. Complete blood studies were determined prior to and during acetazolamide administration. The survival of autotransfused $Cr^{51}$-labeled erythrocytes were studied in the two infants. Acetazolamide was given the infants for several weeks prior to labeling of the red cells and then discontinued during the radioactive study. The diagnosis of sickle-cell anemia was determined in all patients by electrophoretic studies, alkaline-resistant determinations, and sickle-cell preparations.

In the adults there was no effect of acetazolamide on hemoglobin levels and hematocrit. The effect on the reticulocyte was variable; two patients had a drop, one, a rise. The hemoglobin in one infant remained stable while on acetazolamide, but, in the other, it rose 2 gm. %. The response of the reticulocyte count was variable in these infants.

Because acetazolamide is rapidly excreted within an eight-hour period, it was felt that only one labeling procedure was necessary. There was no

* By invitation.

alteration in the erythrocyte survival curve (4 days and 10 days, respectively) in the two infants when acetazolamide was discontinued. In the former patient the survival time had been seven days when determined six months prior to the test period on acetazolamide.

### 33. The Treatment of Ammonia Toxicity in Erythroblastosis Fetalis

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A study was made of the blood ammonia values in erythroblastotic infants. A series of patients with persistent central nervous system symptoms showed elevated and progressively rising blood ammonia values in the presence of relatively low bilirubin and heme levels. They were treated with arginine, sodium glutamate, L-glutamine, and arginine glutamate in an effort to correct their difficulties. A series of cases will be presented demonstrating the responses of these patients to specific therapy with return of blood ammonia levels to normal and coincidental clinical improvement. The effect of arginine in increasing urea synthesis will be shown. Electroencephalographic records reveal a correlation of high blood ammonia levels with abnormal records.

The possible mechanisms involved in this process will be discussed, together with the suggestion of a therapeutic procedure for erythroblastotic infants who show cerebral symptoms.

### 34. A Metabolic Neurovisceral Disorder with the Accumulation of an Unidentified Substance: A Variant of Hurler's Syndrome?

JOHN M. CRAIG, JOHN T. CLARKE,* and BETTY Q. BANKER,* Harvard

A girl born to a mother with one normal offspring displayed at birth a large liver, a large spleen, and ascites. Exploration for intestinal obstruction at 20 hours of age revealed a malrotation of the small intestine and yielded 500 cc. of ascitic fluid.

At 10 days of age, the liver was 6.0 cm. and the spleen 4.0 cm. below the costal margin. Bilateral inguinal herniae were present. The reflexes were normal, though restricted motions of the neck, hips, and knees were present.

The positive laboratory findings included a 1+ urine albumin, anemia with nucleated erythrocytes in the peripheral blood, and vacuolated mononuclear cells and histiocytes in the bone marrow aspiration. Roentgen examination revealed abnormalities of the dorsal vertebrae, bilateral dislocation of the hips due to underdeveloped acetabula, and widened shafts of the long bones with coarse trabeculation. The child was discharged after the performance of a liver biopsy. She pursued a gradual and relentless downhill course at home, dying at the age of 3½ months.

At autopsy, the infant was marasmic. Forty cubic centimeters of clear fluid was present in the abdomen; the liver was twice and the spleen three times normal weight. The brain was 100 gm. below normal weight for the age and was firmer than normal. There was an especially increased consistency of the cortical spinal tract area.

The Kupffer cells in the liver biopsy, and at autopsy most of the reticuloendothelial cells, were swollen and had a faint eosinophilic cytoplasm. A striking finding was the swelling of the cytoplasm of the glomerular epithelium in the kidney, which gave a similar staining reaction. In the cerebrum, brain stem, and spinal cord, both neuronal and glial cells had swollen pale cell bodies. This change was accompanied by a breakdown in myelin where myelination had taken place. Histochemically the affected cell cytoplasm was found to be periodic acid-Schiff (PAS)-positive. This reaction persisted after methylation and after digestion with amylase, pectinase, glucuronidase, and hyaluronidase. The PAS reaction was abolished by acetylation. The affected cell cytoplasm was not metachromatic even with Lindsay fixation, but metachromasia became marked in the affected cells after sulfonation. The Hale and alcian blue stains for acid polysaccharides were strongly positive. Except for the macrophages in the perivascular areas near myelinated structures in the brain, no positive reaction for lipids could be obtained. Stains for protein or aromatic amino acids gave only faintly positive reactions in scattered numbers of the otherwise uniformly affected cells.

Chemical extraction was performed on the fine-ground frozen liver (and a control sample), with use of an initial extraction with 18% formaldehyde. The supernatant was treated with 5% trichloracetic acid, and in each case a precipitable and non-precipitable fraction was obtained. These fractions were then analyzed for total amino acids and for monosaccharides, hexosamines, and n-acetyl-neuraminic acid. No significant differences among these moieties could be detected in the abnormal and control livers. Though no significant differences were present in the chemical determinations, the original extraction procedure may not have eluted the essential material, since it is well fixed in the tissue by formalin fixation.

Though the association of accumulation of mucopolysaccharides with bony deformity and the involvement of the brain as well as the viscera suggest the diagnosis of Hurler's syndrome, the absence of metachromasia in the cells and the involvement of the glomerular epithelial cells exclude this case from that category.

* By invitation.

### 35. Respiratory Illness in Children Simultaneously Infected with Hemadsorption Virus Type 1 and a Newly Recognized HeLa Enterovirus (HE)

HENRY G. CRAMBLETT, JULIUS A. KASEL,* and JOHN P. UTZ,* Iowa and the National Institute of Allergy and Infectious Diseases

This report describes etiologic studies of an outbreak of undifferentiated respiratory disease which occurred in a children's convalescent hospital in June, 1956. From the washings of anal swabs of ill children, a cytopathic agent (HE virus) was isolated in HeLa-cell cultures. Serologic and virologic studies indicated that this virus was not related to previously described viruses and that it had the properties of an enterovirus. Studies of serums collected from patients during acute and convalescent phases of illness showed increases in antibody titers against HE virus, which was confirmation that infection with the agent had occurred. This virus was never isolated from control children who did not become ill. In order to rule out concomitant infection with another virus, the serums of the patients were tested for antibodies against influenza viruses Types A, B, and C; adenoviruses, and Types 1 and 2 hemadsorption viruses. These studies were limited by the quantity of serum available for study and were restricted to consideration of viruses that might cause illness similar to that observed in this outbreak.

In several serums from ill children, antibody responses were such as to suggest simultaneous infection with Type 1 hemadsorption virus. This virus is one of two recently described agents which do not regularly cause cytopathic changes in cell cultures, especially on primary isolation, but whose presence can be detected by the hemadsorption technique of Shelokov. After more than two years of storage at —20 C, attempts were made to isolate Type 1 hemadsorption virus in monkey-kidney-cell cultures. In only one instance was a positive culture obtained. However, it has been observed that this virus is poorly preserved at this temperature. This may explain the inability to obtain additional isolations of this virus.

It is impossible from these data to determine which of the viruses, if either, caused the outbreak of illness. There would be support for the speculation that the clinical illnesses were the result of simultaneous infections with HE virus and Type 1 hemadsorption virus. Only additional studies and observations will establish the relationship between infection with HE virus and human illness.

* By invitation.

### 36. Studies on the Function of the Adrenal Cortex in Newborn Premature Infants

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Introduced by Vincent C. Kelley

In an attempt to gain information concerning the function of the adrenal cortex during the newborn period, the 17-hydroxycorticosteroids (17-OHCS) were determined by the method of Reddy on 237 twenty-four-hour urine samples which were obtained from 79 premature infants. Insofar as possible the patients were unselected, consecutive admissions to the premature nursery. The first specimen was obtained as soon after birth as possible, the second at one week of age, and subsequent specimens at weekly intervals thereafter. Of the infants studied, 45 had completely uneventful courses, 11 experienced moderate to severe clinical difficulty, and 23 died. Specimens also were obtained from eight normal term infants during the first week of life.

In a previous report concerning 21 of the present group of 45 normal premature infants it was shown that the mean urinary excretion of 17-OHCS was very low at birth and was significantly higher by 2 weeks of age and again significantly higher at 1 month of age than it was at 2 weeks of age. However, even at one month of age the absolute values remained low in comparison to our values for normal adults. It also was shown that there was no relationship between the 17-OHCS excretion and either sex or birth weight. When the entire group of 45 normal infants was evaluated the results were the same.

A comparison between the *mean* urinary excretion of 17-OHCS of normal premature infants and those prematures who survived a difficult course revealed that no significant difference was detectable during the first three days of life but at both 1 week and 2 weeks of age the urinary 17-OHCS excretion of the abnormal group was significantly ($P<0.001$) less than that of the normal group; thereafter no significant differences were found. It is noteworthy that although in most instances the decrease in urinary 17-OHCS excretion was coincident with clinical distress in some cases it preceded recognizable clinical signs of difficulty.

In the group of patients who died the mean urinary excretion of 17-OHCS at birth was significantly ($P<0.01$) lower than that of the normal group and remained significantly ($P<0.001$) lower at 7 and at 14 days. In the patients who died no significant differences were found between the mean values at the various ages studied, and so these patients as a group exhibited a low flat urinary 17-OHCS excretion curve.

When the group of patients who had difficulty were compared with the group who died, no sig-

nificant differences were found between the mean values of urinary 17-OHCS at 0-3, 4-8, or 9-14 days of age. Thereafter the patients who survived exhibited a substantial and continued increase in urinary 17-OHCS.

At birth, the mean excretion of 17-OHCS by full-term infants was significantly ($P<0.01$) greater than that of newborn premature infants, but no significant difference was found when similar comparisons were made at one week of age. When the mean excretion of 17-OHCS by term infants at birth was compared with the mean excretion by the same infants at one week of age, no significant difference was found.

These data, plus our previously reported findings concerning the similarity of the half-life of hydrocortisone from birth through 15 years of age, suggest that normally the adrenal cortex is functional at birth and this activity increases significantly with age, that at birth the adrenal cortex is significantly less active in premature infants than it is in term infants but by one week of age this difference no longer exists, that a decline in adrenal cortical function can precede the onset of clinical distress in premature infants and therefore must be given consideration as a possible primary cause, and that the degree to which a premature infant's adrenal cortex is able to function at birth may be of great importance in determining whether the infant survives.

### 37. Serum Bilirubin Studies in Newborn Negro Infants

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Introduced by Roland B. Scott

Previous studies have indicated that bilirubinemia in neonatal life may be influenced by several factors, such as period of gestation, race, type of delivery, maternal complications, and administration of vitamin K.

The purpose of this investigation was to establish normal serum bilirubin values under the conditions of delivery and management which obtain in this hospital. Two groups were studied. The first consisted of 37 normal full-term infants who were followed from birth until discharge from the hospital, at the age of 3 days. These infants received 5 mg. of vitamin K intramuscularly on admission to the nursery. Daily serum bilirubin determinations were done on capillary blood.

Inasmuch as it was apparent that the majority of infants in this series had not passed the peak concentration by the third day, another group of 30 normal newborn infants were studied. The dose of vitamin K given to this group was 1 mg. The group included 18 infants delivered by cesarean section and 10 infants weighing between 2,000 and 2,500 gm. Daily serum bilirubin determinations were done from birth to 5 or 7 days. Blood groups were determined on mother and infant. Possible blood-group incompatibility was present in only four mother-infant pairs. The maternal S. T. S. was negative in all cases. The mean serum bilirubin concentration values from birth to 7 days were as shown at the bottom of page (milligrams per cent).

### 38. The Effect of Face Masks on the Respiration of the Newborn Infant

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WILLIAM H. TOOLEY,* and KURT WEISSER,*
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Introduced by Francis Scott Smyth

A number of workers have studied the volume and frequency of respiration in the newborn infant, and it is disturbing to find that the results obtained show marked discrepancies according to the method used. For example, in one study with the baby plethysmograph, an approximate average minute volume of 600 ml. and a frequency of 30 per minute is contrasted with a volume of 400 ml. and a frequency of 50 per minute when face masks and valves were used.

As we could find no record of a direct comparison being made, we have studied the effects of three different sizes of face masks on the respiration of babies who were sleeping quietly in the body plethysmograph. It is apparent that with a mask there is an increase in the minute volume of respiration, which is related mainly to the added dead space.

### 39. Aldrich Syndrome in Twins

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The occurrence in twins of the syndrome of thrombocytopenia, eczema, and infection in infants,

* By invitation

| Age, Hr. | Cord | 0-12 | 13-24 | 25-48 | 49-72 | 73-96 | 97-120 | 121-144 | 145-168 |
|---|---|---|---|---|---|---|---|---|---|
| First group | | 3.0 | | 6.0 | 8.1 | | | | |
| Second group | | | | | | | | | |
| All cases | 2.8 | 3.1 | 4.3 | 6.2 | 7.1 | 6.3 | 6.0 | 4.9 | 3.5 |
| Full-term (cesarean section) | 2.0 | 2.1 | 4.6 | 5.9 | 6.6 | 5.9 | 5.1 | 3.7 | 2.6 |
| Premature | — | — | 3.9 | 5.9 | 8.0 | 7.9 | 7.1 | — | — |

described by Aldrich, in 1954, gave us an opportunity for controlled experiments concerning the pathological physiology of this syndrome.

These 3½-year-old twins were dependents of a British major with a family history including the deaths of two male siblings from the same disorder. In addition to thrombocytopenia (20,000 to 50,000 platelets per cubic millimeter) and spontaneous bleeding, including ecchymoses and epistaxis, these infants were violently allergic. One spoonful of peas would produce a bloody diarrhea. Turnips produced swelling of the joints. Touching the skin with an unbroken egg produced wheals. Even goat's milk provoked immediate vomiting and wheezing. Although these infants were very susceptible to infection they did not have chronic otitis media. Steroids were partially effective in controlling allergic manifestation but had very questionable effect on controlling thrombocytopenia.

Investigations of immune mechanisms were unrevealing as manifest by normal γ-globulin levels, normal response to antigens, and the absence of platelet agglutinins. Bone marrow examinations showed normal megakaryocytic activity. If anything, these children seemed hyperimmune, since one of them developed a hemolytic anemia following a compatible transfusion. He later had a markedly positive direct Coombs test with both an isoagglutinin and an autogglutinin.

Porphyrin metabolism, serotonin metabolism, and epinephrine measurements were normal. Attempts at virus isolation were unsuccessful.

As would be expected, scratch intradermal and passive transfer tests were positive to a myriad of antigens.

The usual hypoallergenic environment and diets had some beneficial effect on the eczema but no influence on the thrombocytopenia.

Attempts were therefore directed to protein digestion, absorption, and metabolism. To rule out poor protein digestion or a missing intrinsic factor, material obtained by duodenal aspiration from healthy donors was fed in large amounts by mouth without benefit. To rule out the possibility of peptone or peptide absorption one twin was fed a formula similar to milk, composed of human albumin, corn seed oil, and glucose. The other infant received a formula of synthetic amino acids, corn seed oil, and glucose. Positive nitrogen balance was demonstrated on both these formulas. Although both twins had manifest minimal allergic phenomena on their regimen, the thrombocytopenia was not affected. Attempts to demonstrate an abnormal peptide in the serum were also unsuccessful.

Serum amino acids were determined on four occasions by column chromatography (Dr. Levenson). Very low values were found for glycine, taurine, guanido-acetic acid, leucine, phenylalanine, tyrosine, histidine, and proline. There was no difference in the amino acid levels, whether the children were on synthetic diets or their regular hypoallergenic diets, consisting of rice, potatoes, butter, and various amino acid hydrolysates. This depression of certain amino acids was also present in another set of male twins in the family who did not have the disorder and in the mother. The father and daughter had normal plasma levels.

### 40. Influence of Carbohydrates on Cerebroside Deposition in the Brain and Spinal Cord of the Guinea Pig

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Studies on the chemical composition of mammalian brain and spinal cord have been limited to the mouse, rat, and human infant, the latter two species receiving far less investigation than the first. A positive correlation between the cerebroside content of the brain and the lactose content of the milk of several mammalian species has been demonstrated. Platt (*Brit. M. J.* 1:179, 1955) has speculated on the association between dietary lactose and the myelinization process. Testing a possible relationship seemed feasible through a study of cerebroside deposition in newborn guinea pigs fed diets containing different types of carbohydrate.

A standard commercial guinea pig chow containing 44% carbohydrate in the form of starch and molasses and a semisynthetic diet containing 53% lactose were fed to two groups of guinea pigs. The cerebroside content of spinal cord, brain, and liver was calculated from determination of the galactose content of a specific lipid fraction isolated from these tissues.

Chow-fed guinea pigs under 3 days of age had significantly lower levels of brain and spinal cord cerebrosides than animals 5 to 23 days of age. Spinal cord cerebrosides for guinea pigs 10 days of age and younger were significantly less than for animals 18 or more days old.

Cerebroside values for the spinal cord and brain of guinea pigs fed the lactose-containing diet were comparable to or slightly lower than the values obtained from the chow-fed group.

No deposition of cerebrosides was detected in the liver or C. N. S. of the fetal guinea pig before 30 days of fetal life. However, cerebrosides accumulated in the liver prior to their appearance in C. N. S. The rate of deposition of cerebrosides in utero was not influenced by maternal diet.

This experiment indicates that (1) lactose is without effect on the cerebroside content of the spinal cord and brain of the fetal as well as newborn guinea pig and (2) cerebroside deposition occurs in the C. N. S. of the guinea pig fetus after 30 days' gestation and continues to increase for

* By invitation.

18 days after birth. As a corollary finding, the guinea pig, unlike the rat, tolerates a high-lactose diet without developing diarrhea or cataract. Furthermore, this diet was found to be adequate for reproduction through five generations.

### 41. Studies of Renal Function in Vitamin D-Refractory Rickets

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These studies were undertaken to evaluate the role of the kidney in the pathogenesis of the hypophosphatemia and the relative hyperphosphaturia characteristic of vitamin D-refractory rickets. Two principal hypotheses have been proposed to explain these findings: (1) a primary alimentary defect in calcium absorption leading to secondary hyperparathyroidism in an effort to maintain serum calcium homeostasis and (2) a primary intrinsic renal tubular defect in the mechanism for phosphorus transport.

These theories were examined in 10 patients (8 children and 2 adults) with vitamin D-refractory rickets. Three patients (two adults and one infant) had received no prior vitamin D therapy, and the others had been off vitamin D for four to six months prior to study. Discrete renal function studies in all patients revealed no abnormalities. Tubular excretory capacity for PAHA was normal. The tubular conservation of bicarbonate, the excretion of ammonia following chloride loading, and the excretion of titratable acid following phosphate loading were all normal.

Each patient was deprived of dietary phosphorus, and tubular function was studied before and during phosphate loading. Tubular phosphate conservation and maximum rates of reabsorption were normal in the seven patients who had received some vitamin D therapy and decreased in the three patients who had received no treatment.

The influence of parathyroid activity on tubular phosphate transport was evaluated by a modified calcium-infusion test. These studies were compatible with the presence of parathyroid hyperplasia in the untreated subjects and were normal in the patients who had received prior vitamin D therapy.

These studies suggest that an intrinsic renal tubular defect in phosphorous handling is not the primary abnormality in vitamin D-refractory rickets.

### 42. Identification of the Galactosemia Heterozygote

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Introduced by Robert Ward

The ability to detect carrier of various inherited traits is a need in genetic counseling. Progress has been made in several areas, as exemplified by the ability to recognize the heterozygote in a number of the hemoglobinopathies, in cystinuria, and in phenylketonuria. Galactosemia, a metabolic disorder of genetic origin, is known to result from a deficiency of a specific enzyme (galactose-1-phosphate-uridyl transferase) necessary for the conversion of ingested galactose to utilizable glucose. The frequent occurrence among siblings of affected children and the equal distribution among sexes indicate transmission as an autosomal recessive. Methods used for confirming the diagnosis of galactosemia have not been entirely successful in identifying the heterozygous state. Galactose-tolerance tests and the more recently employed assay of erythrocyte transferase levels have suggested a statistical difference between known carriers and normal persons. However, examination of published data forces the conclusion that overlap between ranges makes identification of the heterozygote uncertain. Some important modifications in the transferase assay now have resulted in a more satisfactory procedure. The present study is an application of this method to over 200 persons, including the members of 13 galactosemic families, their relatives, and normal controls. The galactose-1-phosphate-uridyl transferase values obtained could be separted into clear-cut groups. The mean values found (expressed as micromoles of UDPGlu consumed per hour per milliliter of red cells) are summarized as follows: homozygote, mean 0.1, range 0-0.6; siblings (assumed heterozygote), mean 3.1, range 1.1-4; siblings (assumed normal), mean 6.1, range 5.0-7.1; parents, mean 2.9, range 1.1-4.0; relatives (assumed heterozygote), mean 2.8, range 1.9-3.9; relatives (assumed normal), mean 6.1, range 4.2-7.6; normal controls, mean 5.9, range 4.2-8.1. It has been shown that heterozygotes have a transferase level clearly intermediate between affected and normal persons. The lack of overlap is evident. These results show that the improved enzymatic method can be applied to detection of parents likely to give birth to galactosemics. The concept of galactosemia being inherited as a simple autosomal recessive characteristic has been confirmed.

### 43. The Effect of Sodium Glucuronate and Glucuronolactone on Bilirubin Conjugation

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The present study was undertaken to evaluate the relative physiological role of glucose, glucuronolactone, and free glucuronic acid as precursors of the glucuronate moiety of conjugates. Fasting adult mongrel dogs were anesthetized with intravenous pentobarbital (Nembutal); a catheter was placed in the bladder and in each femoral vein,

* By invitation.

and the common bile duct was cannulated. Control specimens of urine, bile, and venous blood were obtained. Glucose, glucuronolactone, or sodium glucuronate, 220 mg. per kilogram of body weight, uniformly labeled with $C^{14}$ was given over a one-hour period by intravenous infusion. An intravenous infusion of 15 mg. per kilogram of body weight of bilirubin (Pfanstiehl) was started simultaneously and given over a 20-minute period. Bile and urine were collected for a three-hour period from the start of the infusion, and serial blood specimens were obtained during this period. Each of the three experiments was repeated a second time.

Of the labeled material injected, 0.046% was recovered from the bile of the dog given sodium glucuronate; 0.35%, from the bile of the dog which received glucuronolactone, and 1.88%, from the dog given glucose. In the dog given sodium glucuronate, 35.1% of the injected material was recovered from the urine; 36.7% of the injected label was recovered from the dog which received glucuronolactone, and 14.1%, from the animal given glucose.

Aliquots of each bile specimen were coupled with an excess of diazotized sulfanilic acid, the azo pigment extracted with butanol and separated by ascending chromatography on S & S No. 598 paper with use of 3:1:1 ethyl methyl ketone, n-propionic acid, water as developer according to the procedure of Schmid. The chromatograms were dried and scanned in a Forro gas-flow radiochromatogram scanner for radioactivity. No label was detected at the spot corresponding to bilirubin in the case of the animal given sodium glucuronate; in the case of the animal given glucuronolactone slight radioactivity was present, and in the animal given glucose more radioactivity was detected.

In the past, Danoff et al. (*Science* 127:759, 1958) have claimed a considerable measure of success in the treatment of hyperbilirubinemia in the newborn with the oral or parenteral administration of large doses of glucuronolactone or in particular sodium glucuronate. From the present data, it would appear that sodium glucuronate does not influence significantly the conjugation of bilirubin to bilirubin glucuronide.

### 44. Electrocardiographic Evaluation of Pulmonic Stenosis

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Patients with pulmonic stenosis and intact ventricular septum often have similar symptoms, physical findings, and roentgenographic manifestations despite differences in severity of stenosis. This poses a problem in management, particularly in selection of patients for cardiac catheterization in anticipation of surgery and in evaluation of postoperative results. A study was undertaken to determine whether electrocardiography fulfills the need for a simple tool to reflect reliably the right ventricular burden.

Analysis was made of electrocardiograms taken close to the time of pressure recording in 65 patients whose right ventricular systolic pressures ranged to 240 mm. Hg. Twenty-seven had mild stenosis (pressure below 80 mm. Hg), twenty-one had moderately severe stenosis (pressure 80-140 mm. Hg), and seventeen had severe obstruction (pressure over 140 mm. Hg). Nine additional records from autopsied cases and 50 published tracings with comparable pressure data were reviewed.

When several features of the electrocardiogram were considered together, it was found that successive increments of right ventricular pressure were associated with additional abnormalities unusual in patients with milder degrees of obstruction. It was possible then to rank the signs of right ventricular hypertrophy merging into "strain" in order of increasing selectivity for detecting severity of stenosis.

When the only changes in the electrocardiogram consisted of right-axis deviation, a predominant R or R′ wave of less than 10 mm. in $V_1$ and the right arm lead, the right ventricular systolic pressure was below 80 mm. Hg.

Reversal of the normal R- and S-pattern in the precordial leads and amplitude of R in $V_1$ greater than 10 mm. were found with pressures of 80-140 mm. Hg.

Signs of right ventricular "strain" or myocardial damage in addition to marked hypertrophy, the displacement to the left of the transitional zone, and high, peaked P-waves indicative of right auricular enlargement were limited to patients with pressures in excess of 150 mm. Hg.

These electrocardiographic criteria were found to be applicable to postoperative patients as well. In 10 such patients the form of the electrocardiogram one year after surgery indicated accurately the right ventricular pressure found at postoperative cardiac catheterization.

Thus analysis of the electrocardiogram is believed to be useful for assessment of degree of obstruction in patients with pulmonic stenosis and normal aortic root.

### 45. Transitional Disease in Infants of Diabetic Mothers

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This report is concerned with the clinical pictures encountered in infants of diabetic mothers during the immediate neonatal period. The patient material

* By invitation.

is composed of consecutive live-born infants of diabetic mothers delivered on two clinic services over a two-year period (23 cases) and those seen by one of us in a private pediatric practice over the same period of time (7 cases). The infants were kept under close physician surveillance during their hospital stay.

According to the clinical pictures, the patients were divided into four groups: I. no observed disease (10 cases) and transient symptomatology without a dfinite clinical picture (3 cases); II. cardiopulmonary distress (6 cases); III. central nervous system distress (6 cases); IV. combined cardiopulmonary and central nervous system distress (5 cases).

There were two deaths in this group of patients. One infant included in the cardiopulmonary distress group died on the fourth day of life following repeated apneic episodes. The other infant succumbed to a hospital acquired infection after apparent recovery from the immediate neonatal distress.

The sequence of symptomatology suggests that the infants were undergoing a recovery of the neuraxis following a prior depression. The signs and symptoms can be correlated with a cerebral level of activity and occur in an orderly and predictable manner. The clinical picture of the recovery phases seen in infants of diabetic mothers is similar to that seen in infants with prolonged postnatal umbilical cord pulsations previously reported from this unit. The collective symptomatology has been termed transitional disease of the newborn (Desmond, M. M.; Kay, J. L.; Megarity, A. L., Jr., and Brown, C. A.: Transitional Disease Associated with Prolonged Postnatal Umbilical Cord Pulsation, to be published).

### 46. Erythrocytic Glucose-6-Phosphate-Dehydrogenase Activity and Anemia Due to Infection

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A hereditary abnormality of the red cells has been described in 10%-12% of American Negroes sensitive to primaquine (Beutler). A low reduced glutathione (GSH), GSH instability, and low activity of glucose-6-phosphate-dehydrogenase (G-6-P-D) were demonstrated in these subjects (Beutler and Carson). The same abnormalities were found to be present in the red cells of subjects who developed hemolytic anemia following ingeston of other drugs such as naphthalene and fava beans (Zinkham and Szeinberg).

In this study the relation of low G-6-P-D activity of the red cells and hemolytic anemia induced by severe infections was investigated. The G-6-P-D levels were determined by a method described by Paul Marks based on that of Kornberg and Horecker. Our values for G-6-P-D activity are as follows:

| | ΔO.D/min/gm/Hb. |
|---|---|
| Normal adults & children (37) | 14.2±4 |
| Newborns (6) | 23.0±4.3 |
| Abnormals (all ages) (18) | 4.7±3.6 |

Seventeen patients with severe infections (mainly acute bacterial meningitis) were investigated. Eleven of the seventeen patients had normal G-6-P-D levels and did not develop hemolytic anemia. Of the six patients who developed hemolytic anemia as shown by a drop in hemoglobin of 2 gm. % or more in one week, five had low levels of G-6-P-D. Four of these patients were Negroes, and the fifth one was a white Puerto Rican. Three of them received sulfisoxazole (Gantrisin) along with other antibiotics while they had the severe infection. One Negro Puerto Rican patient who developed hemolytic anemia along with pneumococcus meningitis and who had received sulfisoxzole was found to have normal G-6-P-D levels, as well as seven of his siblings.

The enzyme activity was determined at the time of infection in three patients and up to a year following the infection in three others. Family studies were carried out in vitro in all patients, and other affected relatives were found in two of the families. In vivo studies with sulfisoxazole are in progress in patients who received this antibiotic at the time of their infection to see if this drug alone can induce anemia. Although the number of patients studied is small, the fact that five of six patients who had a hemolytic anemia associated with infection had low G-6-P-D levels seems to indicate that there may be a causal relationship. Further in vivo and in vitro studies are planned to clarify this point.

### 47. A Hemagglutination Test for Staphylococcal Disease

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There is a need for better serological "tools" for the study of staphylococcal disease.

In 1948, Keogh, North, and Warburton reported a phenol-insoluble staphylococcal polysaccharide which could be readily adsorbed onto erythrocytes and so render those cells agglutinable by staphylococcal immune serum. In 1952, Rountree and Barbour noted that cord blood did not contain this antibody—even if maternal blood did. This observation suggested that a test for this antibody might be of diagnostic value in the neonatal period.

We were able to prepare satisfactory antigens from trypticase soy broth cultures of staphylococci by extracting the whole organisms with ether and

* By invitation.

alcohol, with formamide, or by concentrating the supernate from an 18-hour culture by boiling with dilute acetic acid and precipitating with alcohol.

A polysaccharide prepared from an epidemic strain of Staphylococcus by the method used by Julianelle and Wieghard, in 1935, for their skin-test material was found to act in this hemagglutination test essentially as did our other extracts.

When the same staphylococcal extract was compared in a capillary-tube precipitin test and in a hemagglutination test, the hemagglutination test was far more sensitive. For example, a child's serum which gave a titer of 1:4 with undiluted extract in the precipitin test had a titer of 1:160 in the hemagglutination test.

We found, as had Rountree and Barbour, that cord blood was essentially free of antibody to this polysaccharide when it was adsorbed onto human red cells, even when the maternal serum contained considerable amounts of this antibody. We also found that about one-half of all the sera tested from persons over 6 months of age had this antibody in titers of 1:20 or higher. However, among a group of 26 infants under 6 months of age there were only 5 who gave a positive test; 3 of these were thought clinically to have staphylococcal infections, 1 had bronchiolitis, and 1 had unexplained fever. In some instances serial specimens of serum were available and a rise in titer could be demonstrated. However, there were a few infants in this age group who had proven staphylococcal infections who failed to develop these antibodies.

As a result of this survey it is believed that a positive staphylococcal hemagglutination test in an infant under 6 months of age or a rise in titer of this antibody in a person of any age indicates a recent staphylococcal infection. The ease of performance and the sensitivity of the test recommend its inclusion in our diagnostic armamentarium.

### Discussion

DR. JOHN CYRIL PETERSON, Milwaukee: I would like to ask if the authors were able to demonstrate the development of antibody in infants who became carriers without an apparent infection.

DR. C. HENRY KEMPE, Denver: I wonder if Dr. Florman has compared his titers with antitoxin levels, using a rabbit cell system?

DR. RALPH WEDGWOOD, Cleveland: In view of Dr. Smith's paper at this meeting, is this antibody a macroglobulin (19S)? Is that why it doesn't go through the placenta?

DR. A. NUNNERY, Rochester, N. Y.: I should like to ask how specific the antigen is with reference to its reaction with nonstaphylococcic antisera?

We can confirm Dr. Florman's observations in general in regard to the effect of age on the hemagglutination titers.

An additional observation by Dr. Meyer, who has followed about 90 newborns through the first year of life in our well baby clinic, indicated that those infants who had nasal cultures positive for coagulase and staphylococci early in life were the poorest antibody producers. This interesting observation is being further studied.

DR. FLORMAN: Dr. Peterson, we did not test the blood of newborn infants who were only carriers of staphylococci to see whether or not they developed antibodies. We concentrated on proved instances of staphylococcal infection, and even then we could not demonstrate that all developed these hemagglutinating antibodies.

Dr. Kempe, we did not look for antitoxins. You may recall that antibodies for one of the best-studied toxins, the alpha hemolysin, do cross the placenta. Indeed the antitoxin titers are often higher in the normal newborns than they are in the mothers. Since we were looking for a serological test which would be useful as an indicator of an infant's infection, we did not work with antitoxins.

Dr. Wedgewood, I do not know whether the failure of the hemagglutinating antibody to cross the placenta is a reflection of its large size. It is true that not all antibody molecules cross the placenta equally well. Some years ago we presented some observations before this society which indicated that mumps complement-fixing antibody crossed the placenta in titer, while the Streptococcus MG antibody, which we presumed was a different-sized molecule, did not get across very well at all.

Dr. Nunnery, our antigen is not specific for staphylococci. It is not a purified substance and surely contains a number of bacteria-derived antigens. Some of these are probably heterophile-antigens. This may be why red cells sensitized with our antigen also reacted with some antistreptococcal rabbit sera. Among these was one furnished by Dr. McCarty of the Rockefeller Institute. It was the one he had used to detect his recently described polyglycerophosphate antigen, which is common to many Gram-positive bacteria. This antistreptococcal serum gave a very high hemagglutinating titer with red cells sensitized with our staphylococcal antigen, a much better titer than we obtained with some of our antistaphylococcal rabbit sera.

## 48. Equivalence of Protein from Human Milk and Cow's Milk in Promoting Retention of Nitrogen by Infants

SAMUEL J. FOMON, Iowa

Statistical analysis (Hickman, J. C., and Fomon, S. J.: Analysis of Factors Influencing the Variance in Retention of Nitrogen by Infants Less than 6 Months of Age, to be published) of data from

nitrogen balance studies performed by Fomon and May (Fomon, S. J., and May, C. D.: Metabolic Studies of Normal Full-Term Infants Fed Pasteurized Human Milk, *Pediatrics* 22:101, 1958) with infants fed pasteurized human milk as the sole source of calories revealed that age and intake of nitrogen (milligrams per kilogram) were the major variables influencing retention of nitrogen (milligrams per kilogram). Between 1 and 6 months of age the combined influence of age and intake of nitrogen on retention of nitrogen could be described by a regression plane. Two-thirds of the observed retentions of nitrogen of the infants fed human milk fell no further than 17.8 mg. per kilogram per day from the regression plane.

The concept of the regression plane is illustrated by photographs of a three-dimensional model.

Studies similar to those performed with infants fed human milk have been performed with infants receiving two different cow's-milk feedings with content of nitrogen equal to that of human milk (1.1 gm. per 67 Cal. per 100 ml.).

Three infants received one of these feedings (Formula 657) during a total of 154 days of observation and gained weight at a mean rate of 18.8 gm. per day, compared with a mean rate of gain in weight of 24.3 gm. per day for infants fed pasteurized human milk in the previous study. The mean retention of nitrogen in 11 metabolic balance studies performed with these infants was 101 mg. per kilogram per day, compared with a mean retention of 112 mg. per kilogram per day to be anticipated (on the basis of the regression equation) if the infants had been fed human milk. The difference is significant at the 10% level of probability.

Three infants were fed another formula (Formula 1257) from birth until approximately 5 months of age. The mean rate of gain in weight was 27.5 gm. per day. The mean retention of nitrogen in 22 metabolic balance studies was 98.0 mg. per kilogram per day, compared with 93.7 mg. to be anticipated if the infants had been fed human milk.

Additional data are being accumulated with Formula 1257. Results thus far suggest that this formula with concentration of nitrogen equal to that of human milk may be equally effective in promoting retention of nitrogen.

### 49. Survival of Skin Homografts Following Exchange Transfusion for Neonatal Jaundice

R. Fowler Jr.,* W. K. Schubert,* and C. D. West, University of Cincinnati

The phenomenon of acquired immunological tolerance to homografts can be experimentally evoked in certain species by the injection of leukocytes into fetal or newborn animals. It is not yet known, however, whether such tolerance can be induced in human neonates. For the purpose of testing this possibility, exchange transfusions for jaundice of the newborn affords unrivaled opportunities which have not yet been fully exploited.

In this present study homografting of adult skin has been combined with exchange transfusion in five newborn infants with hyperbilirubinemia. Full-thickness homografts of defatted skin, measuring 0.5×1.0 cm., were placed on equivalent surgically created skin defects in close proximity to the umbilicus. Prolongation of graft survival was observed in those babies receiving grafts from unrelated donors of fresh blood. The blood for these exchanges was drawn just prior to use into polyvinyl bags so as to preserve as many viable leukocytes as possible.

All five grafts in this group show survival times in excess of 21 days, and currently the initial graft is still surviving after a period of more than 12 weeks. It has been shown by Peer that human neonates can reject skin homografts in periods not longer than 21 days, a performance which is virtually on a par with adult rates of rejection. This observation has been confirmed by control cases in our own study.

One other observation of interest was that in spite of the large-scale transfer of leukocytes in these infants there was no passive transfer, such as there would be in adults, of delayed cutaneous hypersensitivity to either histoplasmin or P. P. D.

There seems no doubt, from the observations on homografts, that some form of tolerance or immunological paralysis has been evoked by the interference described, although as yet it is too early to evaluate whether this tolerance is complete or incomplete in nature. It is not yet known what possible influence there may be on survival time from the several variable factors peculiar to the clinical situation under investigation, such as prematurity, erythroblastosis, hyperbilirubinemia, or the depressant effect on bone marrow of the exchange procedure itself. Control studies to explore these relationships are in progress.

#### Discussion

Dr. Andrew Sass-Kortsak, Toronto: I think that these studies are extremely interesting. I wonder whether you have had any opportunity to do the grafting later than a few days after birth.

My other question is in relation to your finding of the extended survival of the mother's tissue. The newborn is probably not able to produce antibodies. Is it possible that you are dealing here with antibodies transmitted from the mother? This seems unlikely. How do you explain then this phenomenon?

* By invitation.

DR. RALPH J. WEDGWOOD, Cleveland: Is the tolerance, if it is such, specific? Were any heterologous grafts, using skin from a donor different than the donor of the blood, attempted after replacement transfusion with viable cells?

DR. VICTOR C. VAUGHAN III, Augusta, Ga.: At my naive level of understanding of this problem I have gained the impression that in some circles immunologic tolerance is felt to be contigent upon extended and continued presence of any potential antigen to which a recipient is acquiring tolerance, and that when this presence lapses, so may the tolerance.

I wonder whether the authors have speculated as to what property in fresh blood may bridge the gap between stored blood and skin antigen. Does the white cell, or any other element in fresh blood, for example, have a capacity for duplication and persistence in the recipient which might account for this result?

DR. FOWLER: In reply to Dr. Sass-Kortsak, we didn't attempt grafting any later than the fourth day of life.

In regard to whether the transfer of antibodies from the mother to the child would influence the graft survival, it just seems unlikely on the basis of the little we know. The antibodies which we know can be transferred are the ones in the γ-globulin system but so far nobody has been able to demonstrate by classic immunological techniques any circulating antibodies to homografts. I think, therefore, that it is still in the realm of speculation as to what effect maternal transfer of antibodies would have on the survival time.

Dr. Wedgwood raises the question of specificity which has also occurred to us. This can be tested in two ways: one would be to repeat the procedure with fresh whole blood, as we have done, but to apply the skin from an indifferent donor; the other way of doing it would be with specific donor leukocytes in combination with indifferent and with specific donor skin. In view of the fact that we want to test the leukocytes anyway, we would prefer to wait until we can get a satisfactory sterile preparation of leukocytes, from our local blood bank, which is getting ready to cooperate with us on this project.

Dr. Vaughan raises rather a broad spectrum of possibilities with his question about the relation of tolerance to whatever might be persisting in the host after the exchange transfusion. The suggestion has been made, and some people firmly believe, that for the state of tolerance to exist the phenomenon of cellular chimerism or mosaicism must continue for the graft to be protected. I think a fair statement of the position would be to say that for chimerism to exist a state of tolerance is necessary, but the converse is not necessarily true. Tolerance can be induced with cells which do not persist in the individual, but this still leaves open the possibility that tolerance may be due to the persistence of antigenic material. This comes very close to one of the oldest controversies in immunological theory about the persistence of antigens in the host, and I am the least competent person here to discuss that one, but there is still a possibility, as Dr. Vaughan suggests, that persistence of some antigenic material transferred in the blood or in the leukocytes is responsible for the prolonged survival of these skin grafts.

## 50. Studies of the Pathogenesis of the High Renal Clearance of Phosphate in Hypophosphatemic Vitamin D-Refractory Rickets of the Simple Type

DONALD FRASER, JOAN M. LEEMING,* E. A. CERWENKA,* and K. KENYERES,* Toronto

A commonly accepted explanation for the hypophosphatemia characteristic of vitamin D-refractory (vitamin D-resistant) rickets attributes the low serum inorganic phosphorus concentration to a primary defect in the ability of the renal tubule to reabsorb phosphate from the glomerular filtrate.

Observations by ourselves and other investigators were inconsistent with this thesis and led us to study the function of the renal tubules in (*a*) two children with untreated hypophosphatemic vitamin D-refractory rickets of the simple type (no glomerular insufficiency, acidosis, amino-aciduria or cystine storage) and (*b*) one child with rickets due to vitamin D deficiency.

Each child, while on a normal intake of calcium and phosphorus, received, for a period of 12 to 20 hours, a continuous intravenous infusion of calcium gluconogalactogluconate sufficient to establish a moderate increase in the concentration of serum calcium.

In the two children with vitamin D-refractory rickets of the simple type, the excessive urinary output of phosphorus diminished rapidly to a negligible level, which persisted throughout the calcium infusion. At the same time, the serum phosphorus concentration rose, although not quite to normal, and mineral deposition was demonstrated radiographically in the rachitic cartilage. Since no alteration occurred in glomerular filtration during this striking alteration in urinary phosphorus excretion, it was concluded that, during hypercalcemia, the tubules possessed the ability to reabsorb filtered phosphate with great efficiency.

A comparable response occurred in the infant with untreated vitamin D deficiency upon administration of calcium. A significant decrease in the urinary excretion of phosphorus was observed when the serum calcium concentration was increased from 7.2 mg/100 ml. to 9.8 mg/100 ml.

* By invitation.

Since parathyroid hormone increases renal clearance of phosphate and since there is considerable evidence that hypercalcemia suppresses normal parathyroid function, it seemed desirable to investigate parathyroid function further in vitamin D-refractory rickets of the simple type. Accordingly, both patients were given calcium intravenously in gradually increasing, then decreasing amounts for periods of six days. Urinary phosphorus output varied inversely with the rate of calcium infusion; as the rate increased, and while the serum calcium concentration remained within the physiological range, the urinary phosphorus gradually decreased from the initial high to an extremely low level. At the time of maximum calcium infusion, and of minimum urinary phosphorus excretion, parathyroid hormone (Parathormone) (200 U. S. P. units) was injected subcutaneously. The latter was followed temporarily by a rapid increase in phosphorus output, in each case to a level considerably in excess of that observed prior to administration of calcium.

It is concluded that the renal tubules of the patients with vitamin D-refractory rickets of the simple type possessed the potential for efficient phosphate reabsorption, as did the tubules of the patient with vitamin D deficiency. This characteristic is not readily reconciled with the postulate of a primary abnormality in the function of the renal tubules but fits more readily the thesis that the characteristically high renal clearance of phosphate in vitamin D-refractory rickets of the simple type is the result of secondary hyperparathyroidism, the cause of which may prove to be defective intestinal absorption of calcium.

### 51. Potassium, Mitochondria, and Mercury Compounds

J. L. Gamble Jr., R. L. Scott,* and O. Reiss,* Johns Hopkins

Attempts have been made to learn something about cellular manipulation of electrolytes by observations not of the whole cell but of a given part, in this instance the mitochondria.

Mitochondria are important to processes of active transport in that they are essentially the dynamos, the chief source of energy supply in the nucleated cell; 90% of the energy available from glucose is both released and conserved by the oxidative reactions. Dinitrophenol, which disrupts oxidative phosphorylation, the mitochondrial energy conservation mechanism, has been shown to be an effective inhibitor of many examples of active transport as they have been observed in the nucleated cell.

Mitochondria are also of interest in studies of active transport, owing to a capacity to attract and to bind certain ions in rather specific ways. Potassium is bound on the mitochondrial matrix by a site that permits exchange with radioactive potassium in the media of incubation. This binding site will not accept sodium. As reported previously (*J. Biol. Chem.* 228:955, 1957) it has been possible to demonstrate relationships between the characteristic of potassium binding and the activities of the enzymes of oxidation. These enzymes are imbedded in and actually contribute to the substance of the matrix to which the potassium adheres. Under a number of different conditions a close correspondence has been noted between the capacity of the mitochondria to bind potassium, on the one hand, and to implement oxidative phosphorylation on the other.

This specific binding of potassium does not, however, appear to be an integral part of a mechanism for "pumping" potassium into the cell. When radioactive potassium was injected intravenously into rabbits, the free intracellular potassium of the liver and kidney cells became labeled prior to the introduction of radioactivity into the bound mitochondrial fraction. It is noted further that mitochondria of liver cells are located centrally, and they do not appear to be associated with the activities of the membrane. In striking contrast, however, electron microscopy of renal tubule cells (Rhodin: *Am. J. Med.* 24:661, 1958) has demonstrated a remarkably close approximation of mitochondria to the basement membrane. This positioning strongly suggests some sort of participation in membrane transport activity despite the negative evidence with respect to potassium.

From a laboratory viewpoint, studies with mitochondria afford a valuable opportunity for direct examination of factors that control reactivity between electrolytes and a known unit of subcellular matrix. Observations have been made of the effects of inorganic mercurials upon the characteristics of potassium binding. It has been found that additions of 4μg.-8μg. of mercury per milligram of mitochondrial nitrogen will evoke a fivefold stimulation of the rate at which bound potassium exchanges with media potassium. Larger amounts of mercury destroy the binding site and, concomitantly, the capacity to esterify inorganic phosphate. Presumably the effectiveness of the mercurial compounds is due to mercaptide formation with essential sulfhydryl groups. Additions of excess quantities of a sulfhydryl reagent, such as thioglycolate, will remove the mercury from the mitochondria and will reverse the effects upon the potassium exchange rate. Organic mercurials, such as the commonly used diuretic agents, were found to be equally potent with regard to oxidative phosphorylation, but amounts 5-10 times greater were required for the effects upon potassium binding. P-iodosobenzoate and iodoacetamide, agents that oxidize and alkylate sulfhydryl groups, respectively, affected potassium binding and

* By invitation.

oxidative phosphorylation only at relatively high concentrations.

With regard to the organic mercurials, no correlation could be established between effectiveness of various compounds upon potassium binding and potency as a diuretic agent. Observations of potassium binding characteristics of mitochondria isolated from different tissues have not, as yet, brought to light important differences that could be attributed to the particular organ or to the particular function of the organ.

## Discussion

Dr. Donald B. Cheek, Dallas, Texas: Mercury has been used in medicine for centuries and it would seem that we have much still to learn concerning its actions. During the last 10 years, at intervals, we have been investigating the nature and mechanisms in infantile acrodynia, or pink disease. During the course of studies concerning the effect of calomel on the body composition of the rat we found an increase in body potassium content—besides the losses of Cl and Na. Dr. Gamble's paper now provides an explanation for this finding of change in potassium content. However, mercurial diuretic (Mercuhydrin) after injection did not demonstrate a similar change in body K. As I understand it, mercurial diuretics contain a double bond between carbon and mercury and perhaps such agents are weak inhibitors of sulfhydryl groups—while mercuric chloride, the poison is a potent inhibitor. Mercurous chloride (calomel) would seem to inhibit certain areas of enzyme activity more specifically on occasion. Last year at these meetings we presented data showing that calomel could potentiate the action of epinephrine in the body. Recently by direct assay of blood epinephrine we have found that this is undoubtedly true, but coincidental "stress" is necessary to bring the phenomenon to a maximum.

Acrodynia does not resemble acute mercury poisoning, while no case has been reported from the use of a mercurial diuretic. It is only the exceptional child receiving calomel that develops acrodynia. While the general effects of mercury in the body are related to the nature of the mercurial offered, I believe that certain conditioning factors are important also. In acrodynia, for example, preexisting sympathetic over-activity may play a major role in initiating, with mercury, the clinical picture as we know it. Dr. Gamble has shown important changes within the cell, but I have taken the opportunity to discuss some of the actions of mercury on the body as a whole, and one would like to know if all types of mercurials produce the effect on the cell he has shown.

Dr. Robert Winters, Philadelphia: I would like to speak on one point raised by this paper, namely, the relationship between the input of energy and the binding of ions. Dr. R. E. Davies and I have been studying the uptake of radiosulfate by mitochondria of rat kidney cortex; in our system, the actual uptake appears to be a passive phenomenon and follows the classical (Freundlich) adsorption isotherm. The relationship of the input of energy to the uptake process appears to be quite indirect in that it may be sequentially dissociated from the ion movement per se. It would appear that the need for energy is more concerned with the maintenance of integrity of the binding sites; once the sites are "activated," however, ion transport may occur as a purely passive event.

Dr. Solomon A. Kaplan, Los Angeles: In some experiments which we conducted recently, $K^{42}$ was injected into rats and after an appropriate time the specific activities of several tissues were compared with the specific activity of the serum. We found that the specific activity of certain tissues, such as liver, skeletal muscle, and heart muscle, did never exceed 80% of the simultaneous specific activity of the serum. It appears, therefore, that about 20% of intracellular potassium does not exchange freely with extracellular potassium. At the time the experiments were performed, we entertained the possibility that some of this nonexchangeable potassium might be in the mitochondria and, therefore, be unable to exchange freely with potassium outside the cell. What Dr. Gamble has said today throws some doubt on the validity of this hypothesis. However, I should like to ask Dr. Gamble how the specific activity for radioactive potassium in the mitochondria compared with the specific activity of the suspending medium in his experiments.

In connection with the role played by sulphydryl enzymes in the maintenance of the intracellular concentration of potassium, I should like to cite some other experiments which we did when measuring the effect of oxygen at high pressure on the ability of tissue slices to maintain their intracellular concentration of potassium. At atmospheric pressures exceeding three atmospheres of oxygen, brain slices were found to be incapable of retaining the intracellular concentration of potassium. It has been shown previously that oxygen at high pressure exerts its deleterious effects on cells by acting, among other things, on the active sulfhydryl groups of enzyme systems. These observations, in addition to those which Dr. Gamble described today, seem to suggest that enzymes having an active sulfhydryl group are actually associated in the maintenance of concentration of potassium intracellularly.

Dr. C. U. Lowe, Buffalo: Dr. Gamble, did you mean to imply that the sensitivity to mercury was unique in the mitochondria of the kidney, or is

this a general property of mitochondria regardless of tissue of orgin?

DR. GAMBLE: In answer to Dr. Cheek, the sulfhydryl groupings on the enzyme are being referred to with increasing frequency. It seems, of the enzymes studied, that at least half are sensitive to mercury.

We agree with Dr. Winters that there seem to be two phases. If potassium is going to be bound specifically, some sort of energy requirement is in order to build up this binding site. The exchange then occurs more or less passively. This concept is supported by the fact that the temperature coefficient of the exchange is very low. If the temperature is dropped from 20 degrees to zero degrees the exchange rate decreases by a factor of only 0.6.

In regard to Dr. Kaplan's question on the time of equilibration of radioactive potassium, this is a very interesting finding. If 20% is not equilibrated in 48 hours, an important question is raised. Investigators using radioactive potassium to determine total body potassium will certainly have to take this into consideration and make corrections.

We have not worked with high oxygen tensions and I don't think I can add very much to that. Inhibition of mitochondrial enzymes by any means would simulate results obtained with dinitrophenol.

Concerning Dr. Lowe's question, we have tried very hard to look for differences in mitochondria isolated from different tissues—from the liver, kidney, stomach, and intestines—and outside of changes that can be attributed to difference in stability, we have made very little headway. All of the mitochondrias seem to have potassium-binding sites and equal contents of potassium, sodium, and chloride. We don't have much we can say as evidence to place the mitochondria in an active pumping system, other than to say that they are basic units for the supply of energy for the pump.

### 52. A Developmental Study of Children's Ideas About Content and Functioning of the Human Body

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Introduced by Henry L. Barnett

Among pediatricians, there is a growing interest in identifying and in validating ways to help children cope with the emotional aspects of illness. The benefits of appropriate explanations about illness and medical procedures have received much emphasis in this connection. If such explanations are to be meaningful to the patient, they must be geared to his level of understanding and they must take into account his conception about the body. Very little is known regarding the extent and quality of children's knowledge about organic structure and functioning. This study is a pilot investigation of some aspects of children's ideas about the content and workings of their bodies.

Ninety-five children ranging in age from 4 years to 16 years were interviewed. Most of the subjects were patients hospitalized at Massachusetts General Hospital with a variety of acute and chronic illnesses. A standard interview was devised. Included in it were questions such as these: "Tell me as many things as you can think of that you have inside you." "Show me where food goes after you swallow it." "What are the nerves for?" "Are there any parts of you that you could get along without?" In conjunction with the interview, the subjects were asked to locate various organs by filling in an outline of the human body.

Qualitative and quantitative analyses of the data were carried out in order to identify characteristic kinds and levels of information for different age groups. The progression of concepts about the body from preschool age to adolescence was charted. Wide variations were found with respect to the kind and the amount of information children were able to give. Despite this range, it was possible to identify notions that were characteristic of developmental stages. For example, preschool age children tended to report their bodily content as being *food*—an idea that did not appear in later years. Below age 11 years, many subjects located the lungs in the throat and considered their purpose to be "for swallowing." Whereas young children usually considered all their parts indispensable, older ones were able to conceive of living without quite a number of body parts. The location and function of several organs (bladder, liver, intestines, kidneys) were unknown to, or misunderstood by, most children. Reproductive organs were mentioned by only 4 of the 95 subjects. By contrast, most children had some understanding of the nervous system, the skeletal system, and of heart action. Two-thirds of the subjects thought they did not move their bowels as frequently as they should. Children whose responses were unusually immature or markedly advanced as compared with their agemates were frequently found to be emotionally disturbed.

The development of information and conceptions about the body will be presented schematically together with protocols found to be characteristic at the various age levels sampled.

### 53. A Screening Test for Amino-Aciduria

HOSSEIN GHADIMI* and HARRY SHWACHMAN,
Boston

The study of free amino acids in urine is becoming of greater interest because of the associa-

* By invitation.

tion of amino-aciduria with a number of syndromes having genetic implications. Recent reports of amino-aciduria in patients presenting neurologic symptoms and/or mental deficiency may offer a clue in the study of these cases.

The current methods of studying amino acids in urine involves desalting, two-dimensional paper chromatography, and $\alpha$-amino nitrogen determinations. These procedures are expensive, may take from two to four days for completion, and require technical skill and experience in proper interpretation. In view of the normal findings in the majority of specimens submitted for this detailed analysis, it seemed worth while to develop a rapid screening procedure which would safely eliminate a large number of samples for such study.

We propose an easy, rapid, and inexpensive technique for the gross detection of amino-aciduria. As many as 15 urine specimens could be tested in less than two hours. The equipment and reagents necessary are available in most clinical laboratories and consist of scissors, a piece of twine, Petri dish, circular filter paper, micropipet, ethanol, acetic acid, acetone, and ninhydrin. The urine need not be treated previously, nor is a 24-hour collection necessary. Five microliters of native urine of the operator is applied to a section of filter paper and serves as a normal control. Five microliters of each of three other samples to be tested are placed on the three remaining segments of filter paper. The twine is threaded through the center of the filter paper, leaving a small wick approximately 2 in. long. The filter paper is placed on the top edge of the bottom of a Petri dish so that the wick hangs immersed in the solvent (acetic acid-ethanol-water—5:20:15). The solvent spreads from the center of the filter paper horizontally and reaches the edge in approximately one hour. Drying, staining, and interpretation take another 10 minutes. Since the three unknown specimens and the control are prepared under identical conditions, an accurate comparison is possible.

Approximately 400 specimens of urine were studied by this method. Slightly over 200 were from patients in a mental institution and from our neurology clinic; approximately 40, from our urology clinic, and the remainder, from patients in various sections of the hospital having a number of different conditions. The screen test was positive in 31 specimens.

In order to test the reliability of the screen test, a blindfold study was made of 105 twenty-four-hour urines previously examined for $\alpha$-amino nitrogen and by two-dimensional paper chromatography. The screen test detected each of the 16 known or borderline cases of amino-aciduria. Photographs of chromatograms will be shown to illustrate cases of generalized amino-aciduria and of specific amino acid abnormalities.

## 54. Demonstration of Genetic Variants in Carriers of Type A Hemophilia, Suggesting the Presence of an Antihemophiliac Globulin Inhibitor

JOHN H. GITHENS,* University of Colorado
Introduced by Henry K. Silver

No single test has yet been reported that will consistently unmask the hemophilia carrier. In the present study seven hemophiliac families have been investigated by applying the correction technique to the thromboplastin-generation test. The ability of the plasma of the known carrier (female relatives who have given birth to at least one affected male) to correct the thromboplastin generation of the hemophiliac male when added in different proportions (from 5% to 40%) has been compared with the ability of plasma of normal females and normal males to correct the deficiency.

The family history in all seven families gave clear evidence of a sex-linked recessive inheritance of a bleeding disease in males. The "bleeders" all showed defective thromboplastin generation with the defect limited to the $Al(OH)_3$-adsorbed plasma component. Platelet activity, plasma thromboplastin component (PTC) activity, and plasma thromboplastin antecedent (PTA) activity were normal. Therefore, all of the patients have been classified as Type A hemophilia due to antihemophiliac globulin (AHG) deficiency.

In three of the seven families the thromboplastin generation of the male bleeders was corrected by the plasma of normal females. However, in these families the plasma of the known carriers corrected the thromboplastin generation only one-half as well as did that of the normal female. Four sisters were available for study in these three families. The plasma of two of the four showed an ability to correct the defect comparable to that produced by the plasma of the normal female. The plasma of the other two sisters did not correct the abnormality. These findings suggest that the carrier state may be demonstrated by the ability of correct thromboplastin generation.

In the other four families the plasma of known carriers showed an ability to correct the thromboplastin generation test of the affected male that was equal to the correction ability of the plasma of the normal female. Nine sisters of the male bleeders were studied in this group of four families, and the plasma of all nine also showed a significant ability to correct the abnormality. Thus in these four families the female carrier could not be differentiated from the normal female by this technique.

According to present molecular concepts of genetics, the homozygous normal female is considered to have two genes for AHG (one on each of the X-chromosomes), while the heterozygous female hemophilia carrier has one normal gene for

AHG and one that is defective. It is postulated that the hemizygous normal male who has only one gene for AHG on his X-chromosome and none on the Y-chromosome should have AHG activity similar to that of the female carrier, since both have only one normal gene. In comparing the ability of the plasma of the normal male with that of the normal female and the known carrier in the same families to correct the thromboplastin generation of the hemophiliac male it was found that the plasma from normal males in all seven families corrected the abnormality to the same degree as the plasma of the normal female. Thus the normal male and the known heterozygous carrier show the same degree of AHG activity in four of the seven families, while the heterozygous known female carriers in the other three families appear to have much less AHG activity than the hemizygous unaffected male.

Although the results of these studies might indicate that the coagulation defect in three of the seven families is of a different kind, all the usual methods of testing, including attempts at cross correction of plasma of patients in the two groups, would indicate that in all instances the hemophilia was due to AHG deficiency.

The significance of these findings will be discussed, including the possibility that they depend on the presence of a naturally existing AHG inhibitor in the first stage of coagulation. If the gene for this inhibitor is closely linked on the X-chromosome with the gene for AHG, a defect of both genes would produce a heterozygous hemophiliac carrier who would be similar to the hemizygous male. However, a defect of only the gene for AHG would produce a heterozygous hemophilia carrier who would have an excess of AHG inhibitor in her plasma when compared with the hemizygous male.

## 55. Viral Infection, a Possible Cause of Sudden, Unexpected Death in Infants

ELI GOLD, DAVID CARVER,* HANNELORE HEINEBERG,* and LESTER ADELSON,*
Western Reserve

Approximately 30% of all infants between the ages of 10 days and 24 months who die in Cuyahoga County die unexpectedly following either brief, minor illnesses or no apparent illness at all. Postmortem examinations of such children usually reveal minor alterations in the histology of the respiratory tract but none severe enough to be accepted as an adequate cause for death. Several investigators have offered explanations for such deaths, including hypoglycemia, viscerovisceral reflex, and anaphylaxis. In studies of the pathogenesis of viral infections in animals, Webster, Milnick, and Fenner, in particular, have observed that animals given inoculations may die during the incubation period of a virus infection before clinical signs of infection develop. Virus could be isolated from tissues of such animals before any histologic abnormality was detected by usual microscopic examination. With the premise that a similar phenomenon may occur in infants and result in death, a study was undertaken to determine if viral agents could be isolated from the tissues of suddenly dead infants.

Specimens were obtained from 50 infants who died unexpectedly in Cuyahoga County between April and November, 1957. Complete postmortem examinations were performed under the supervision of Dr. Lester Adelson. The home of the child was visited, at which time historical, epidemiologic, and socioeconomic data were gathered. At the same time stool and blood samples and a pharyngeal swab were obtained from family members. All specimens were suitably prepared and inoculated into tissue culture and suckling mice. The virus isolations were as shown in the tabulation below.

* By invitation.

| Case | (Age) | Race | Sex | Mo. of Death | Postmortem Diagnosis | Virus | |
|---|---|---|---|---|---|---|---|
| | | | | | | Source | Characteristics |
| 1 | (2 wk.) | W | F | May | URI | Lung | Spongy degen. in MKC |
| 2 | (1 yr.) | N | F | Aug. | URI | Brain (cortex); stool; stool of contact & throat of another contact (both developed rise in neut. antibody); stool of patient | Type 3 polio<br>Type 3 polio<br>Type 3 polio<br>Type 3 polio<br>Coxsackie A (see text) |
| 3 | (9 ½ mo.) | N | M | Aug. | Myocarditis | Stool; trachea | ECHO, Type ? |
| 4 | (20 days) | W | M | April | CHD; IV & IASD; PDA; cardiac hypertrophy | Spleen | Type 3 polio |
| 5 | (1 ½ mo.) | W | F | Sept. | URI | Stoo | Type 3 polio |
| 6 | (12 mo.) | W | F | July | URI | Brain & other organs | Coxsackie A |
| 7 | (3 mo.) | N | F | June | URI | Brain | Coxsackie A |
| 8 | (13 mo.) | W | M | Sept. | URI | Stool | Coxsackie A |
| 9 | (4 wk.) | W | F | June | Meningomyel. | Brain | Coxsackie A |

The Coxsackie A produced myositis in suckling mice; it is not as yet typed.

These data indicate that 9 of the 50 infants studied had viral infections at the time of their death and lend support to the hypothesis that sudden death in infants may be associated with viral infections in the absence of histologic findings of significance.

### Discussion

DR. C. HENRY KEMPE, Denver: Perhaps the greatest obstacle to rapid advances in research on the viral etiology of sudden death in infants is represented by the commonly encountered morbid pathologist who is disdainful of bacterial and viral studies, does not routinely save autopsy material in the Deepfreeze but quickly submerges all organs in virucidal formalin. Histologic diagnoses are often not possible as the data here shown so well demonstrate. Dr. Adelson is indeed to be congratulated on providing the virologists workable tissue for virus isolation. It would be particularly nice if this paper could come to the attention of pathologists at large because it so well shows that clinically oriented morbid pathology, aware of the contributions to be made by advanced techniques in microbiology and microchemistry, can now enter, though belatedly, the 20th century.

DR. JAMES B. AREY, Narberth, Pa.: I would like to ask whether bacteriologic studies were done in these patients as well as virologic studies and whether there was any concomitant occurrence of positive cultures of bacteria as well as viruses in the babies who died unexpectedly.

DR. GOLD: We did no bacteriologic studies. These have been done by several investigators in the past and have not provided much information. We wanted to use our facilities to look for viruses. Now that some viruses have been found, it might be worth while in future studies to determine if concomitant virus and bacterial infections occur.

DR. WILLIAM A. NEWTON JR., Columbus, Ohio: I think this is a very excellent study and one which needs to be continued with even greater emphasis. I would suggest that it be carried on throughout the year, in addition to the summer months. Since this study was done when enteric viruses are prevalent, I think we have to be very cautious about interpreting these data. This is one of the most baffling problems which we face and it is a common one. Most of the children we see dying with this problem are approximately 3 months of age. I am curious to know why there are so many infants above 3 months in this group reported by Dr. Gold.

DR. GOLD: It is planned to continue this study, gathering material for at least a full year. We will use additional techniques that should enable us to detect a wide range of viruses. The study of material from children who die after trauma or surgery should provide us with control data.

Most of the children in our study are below 3 months of age, Dr. Newton. The general age distribution of our cases is not unlike that reported by Adelson and other observers. It obviously varies with the criteria used to select cases.

DR. FREDERICK C. ROBBINS, Cleveland: I hesitate to keep you people here any longer. You're the faithful guard. I want to find out if the techniques used would be particularly likely to protect the enteroviruses. It is primarily enteroviruses that were detected in this study. I also wonder if Dr. Gold would mention, it was not in his paper, the question of the pathologic findings in the brain of the children with poliovirus and other agents and the titers found in tissues. I know that he has some of these data.

DR. GOLD: You have touched on several very pertinent points.

1. The interval between death and postmortem examination, the method of storing specimens, and the type of isolation methods used all tended to select such hardy agents as the enteroviruses or adenoviruses. It is surprising that no adenoviruses were isolated.

2. It probably was not adequately emphasized in the paper, but all the tissues from which virus was isolated were considered to be histologically normal at the time of postmortem examination.

3. The titers of virus in tissues were comparatively low: $10^{-3}$ or less. This observation is difficult to fit to any hypothesis of "overwhelming virus infection," unless again the methods of this study resulted in a large fall-off in virus titer. It is hoped that information pertinent to this point will be obtained from future studies.

DR. DAVID T. KARZON, Buffalo: It would be interesting to view the isolations in the light of the serotypes of enteroviruses which were extant in the community at the time. Do you have information regarding the rate of isolation of enteroviruses from the alimentary tract of children of this age? Would you compare the serotypes which were recovered from the autopsied cases with those you found in the community?

DR. GOLD: The isolations reported probably reflect the types of agents in the community. We do not mean to suggest there is a specific sudden death virus. We are struck by the observation that in six cases these agents were isolated from *tissue* of dead children. We feel that the proper control for this observation can be obtained only by studying tissue obtained from children dead from known causes such as trauma, that these tissues should come from children in comparable age groups during the same seasons as the study population.

DR. WILLIAM A. NEWTON JR., Columbus, Ohio.: Just one question. You did not differentiate between the groups of patients with and without symptoms of infection. Would you tell about the distribution of virus isolation in these two groups?

DR. GOLD: Unfortunately Dr. Newton, as shown on the slides, we obtained histories from only 34 of the 50 children in the study; of those, 19 had a history of some illness in the 24 hours prior to death. Virus was not isolated from the *tissue* of any of those 19 children but was isolated from the stool and/or pharyngeal secretion of 3 of them.

### 56. Direct Plasma Oxygen Tension Determination in Infants and Adults in Air and in Modified Atmospheres

BRUCE D. GRAHAM, STEVE T. KOEFF,* M. U. TSAO,* C. SLOAN,* and JAMES L. WILSON,
Ann Arbor

We are presenting new data from direct determination of plasma oxygen tension in normal adults and children, in normal infants, and in infants breathing oxygen-enriched and oxygen-low atmospheres. Simultaneous data concerning blood pH, $CO_2$ content, calculated $pCO_2$ and hemoglobin oxygen saturation were determined. The technique used for direct plasma oxygen determination was a modification of the dropping-mercury-electrode technique. It was laborious and time-consuming, and a small volume of data was obtained at great cost, but we believe our findings, though different from preceding reports, are accurate, though much more work is necessary. All data presented in this summary are in abbreviated form. (All samples are arterial.)

* By invitation.

| | pH | $CO_2$, mEq/L. | $pCO_2$, Mm. Hg. Art. | $pCO_2$, Mm. Hg. Alv. | Hgb. $O_2$ Sat., % | $pO_2$ Mm. Hg |
|---|---|---|---|---|---|---|
| Normal children (17), room air | | | | | | |
| Mean | 7.47 | 24.1 | 33 | 39 | 96 | 82 |
| Range | 7.39 | 21.1 | 24 | 36 | 93 | 67 |
| | 7.55 | 28.6 | 45 | 43 | 99 | 98 |
| Normal adults (20), room air | | | | | | |
| Mean | 7.43 | 25.2 | 37 | 38 | 96 | 80 |
| Range | 7.35 | 23.1 | 28 | 33 | 89 | 57 |
| | 7.55 | 27.3 | 46 | 44 | 100 | 96 |
| Normal adults (6), 50% oxygen | | | | | | |
| Mean | 7.42 | 24.5 | 36 | 37 | 100 | 270 |
| Range | 7.37 | 23.3 | 27 | 34 | 98 | 222 |
| | 7.58 | 25.7 | 43 | 38 | 100 | 297 |

A. Twenty normal adults and seventeen normal children between the ages of 6 years and 29 years were sampled in the resting state while breathing room air and then after breathing 50% oxygen for 30 minutes. The results are given in the preceding tabulation.

It is to be noted that the same $pO_2$ range exists for normal children and adults breathing room air.

A comparison of these data to previous studies with smaller groups of adult "controls" reveals a range of normal that was greater and an average arterial plasma $pO_2$ less than previously reported.

B. Data obtained on 15 normal newborn infants 3-50 hours old reveal a significantly lower resting arterial $pO_2$ than obtained in the older control group (other blood gas data are included).

When 50% oxygen was administered to these newborn infants, a definitely lower mean, median, and range of values ($pO_2$) was obtained than in the older control group. These data are abbreviated below:

| | pH | $CO_2$, mEq/L. | $pCO_2$, Mm. Hg | Hgb. $O_2$ Sat., % | $pO_2$, Mm. Hg |
|---|---|---|---|---|---|
| Room air (20% $O_2$) | | | | | |
| Baby A | 7.26 | 18.3 | 39 | 95 | 80±5 |
| Baby B | 7.30 | 20.3 | 38 | 99 | 94 |
| 15% $O_2$ | | | | | |
| Baby A | 7.24 | 18.7 | 41 | 90 | 50±4 |
| Baby B | 7.34 | 20.0 | 36 | 91 | 47 |

| | pH | $CO_2$, mEq/L. | $pCO_2$, Mm. Hg | Hgb. $O_2$ Sat., % | $pO_2$, Mm. Hg |
|---|---|---|---|---|---|
| Normal newborn infants (15), room air (20% oxygen) | | | | | |
| Mean | 7.33 | 19.0 | 34 | 95 | 72 |
| Range | 7.26 | 14.9 | 26 | 87 | 51 |
| | 7.45 | 22.6 | 41 | 100 | 94 |
| Normal newborn infants (8), 50% oxygen | | | | | |
| Mean | 7.34 | 18.6 | 33 | 100 | 159 |
| Range | 7.29 | 16.0 | 27 | 98 | 131 |
| | 7.43 | 20.2 | 40 | 100 | 189 |

Interpretation leads us to consider this difference as being due to (*a*) cardiac shunt or (*b*) pulmonary shunting or diffusion defect. We consider the latter more likely, considering the fact that the $pO_2$ difference in adults and infants in room air is not of great magnitude.

An obvious point of interest is to follow given babies to determine when they will assume the characteristic response of the older child.

C. Because of the emphasis that recently has been placed on the use of 15% $O_2$ for premature

infants, we have obtained data on a small number of paired babies observed first in room air (20% $O_2$) and later 15% $O_2$:

Our conclusions to date are as follows: 1. From our findings the normal plasma oxygen tension is lower and had a wider range than has previously been reported or that is generally accepted. 2. The arterial plasma oxygen tension is lower in normal newborn infants than adults. 3. Plasma oxygen uptake in newborn full-term infants with oxygen-enriched atmosphere is less than in adults. This suggests that the level of oxygen causing harm (retrolental fibroplasia) is even more critical than has previously been believed. 4. Extrapulmonary hemoglobin saturation is possible and often significant in amount. 5. A real and possibly dangerous drop in head of pressure of oxygen being delivered to the tissues is observed with the use of 15% $O_2$.

### 57. Clinical and Biochemical Variations in the Adrenogenital Syndrome

ORVILLE C. GREEN,* CLAUDE J. MIGEON, and LAWSON WILKINS, Johns Hopkins

The clinical syndrome of congenital adrenal hyperplasia has been shown to result from a metabolic defect in adrenal synthesis of hydrocortisone. At least two types of enzymatic defect have been postulated: (1) a deficiency of the enzyme responsible for hydroxylation at $C^{21}$, and (2) an absence of the enzyme responsible for hydroxylation at $C^{11}$ of the steroid nucleus. It has been assumed that each of these enzymatic deficiencies produced a specific form of the syndrome: the nonhypertensive form and the hypertensive form, respectively.

Four patients with the hypertensive form have been studied before and after corticotropin (ACTH) stimulation and cortisone suppression. Fractionation of urinary steroids has been accomplished by chromatography on silica gel, Florisil, and paper. Isolation, identification, and quantitation of various fractions has been performed. Large amounts of the metabolic products of "Substance S" and Deoxycorticosterone were found, as previously described by Eberlein and Bongiovanni (*J. Clin. Endocrinol.* 15:1531, 1955). However, three of the four patients also excreted significant amounts of $C^{21}$ steroids with an oxygen function at $C^{11}$. None of these three patients had typical histories or laboratory findings. In one, the clinical impression was confirmed only by the demonstration of "tetrahydro S" in the urine; urinary 17-ketosteroids and pregnanetriol were not abnormally elevated at the time of study. The other two patients were sisters who did not show virilization until puberty. One had actually experienced female sexual precocity. Hypertension was severe in both, and suppression therapy was successful in reducing the clinical symptoms as well as abolishing the abnormal steroidal patterns of excretion.

The findings in these patients are compared with the patterns found in the classical forms of the disease. "Tetrahydro S" was also found in patients with the nonhypertensive type of the syndrome. The significance of these findings will be discussed.

### 58. Trimethadione (Tridione) Nephrosis: Renal Biopsy and Electron Microscopy Studies

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The opportunity to study an example of nephrosis in childhood caused by a specific etiologic agent is rare. The introduction of renal biopsy plus the availability of electron microscopy may enhance the information gained from observation of such a patient. Accordingly, a 7-year-old boy with the nephrotic syndrome secondary to trimethadione (Tridione) administration was investigated with the use of these newer techniques.

Since the onset of petit mal episodes at 5½ years of age, this patient had been receiving trimethadione in doses of 600 mg. daily. Eighteen months after institution of therapy, generalized edema appeared and a diagnosis of nephrotic syndrome was made at another institution. Trimethadione was discontinued, and prednisone therapy was administered for three weeks. The child was transferred to the Mount Sinai Hospital when generalized convulsions and coma occurred following a rise in his blood pressure.

During the four months of observation, the child remained comatose. Approximately two weeks after transfer, however, a complete diuresis occurred. The serum albumin remained depressed varying from levels of 2.1 to 3.2 gm/100 ml., and the serum cholesterol persisted at slightly elevated values, 300 to 405 mg/100 ml. The blood urea nitrogen levels varied between 8 and 25 mg/100 ml. Albuminuria persisted throughout the entire hospital course. During the postedematous stage urea clearance was 70% of normal; creatinine clearance was 60% of normal. At this point a renal biopsy was performed and demonstrated the following findings.

Microscopic section showed cellular glomeruli with early crescent formation as seen in subacute glomerulonephritis. Electron microscopy also showed increased cellularity, most of the cells being on the inside of the basement membrane (endothelial or intercapillary cells). There were marked alterations of the foot processes with the formation of sheets of epithelial cytoplasm either directly applied to the basement membrane or separated from it by irregular deposits. The basement membrane itself showed increase in thickness and

* By invitation.

variations in density. These changes together indicate severe damage to the capillary walls.

It is hoped that by means of detailed studies such as these performed on children with the nephrotic syndrome caused by a specific etiological agent light may be shed on the pathogenesis of the nephrotic syndrome in general.

### 59. Static Volume-Pressure Relations of Excised Lungs of Infants with Hyaline Membrane Disease and of Other Newborn and Stillborn Infants

IRWIN GRIBETZ,* N. R. FRANK,* and MARY ELLEN AVERY,* Harvard
Introduced by Clement A. Smith

Static volume-pressure measurements were used to compare the elastic behavior of excised lungs obtained from 10 infants with hyaline membrane disease, 5 stillborn infants, and 5 infants dying of other causes during the first four days of life.

To eliminate the effect of variations in lung size, volume was expressed as milliliters of gas per gram of tissue. During deflation from a peak distending pressure of 35 cm. $H_2O$, the lungs of infants with hyaline membrane disease contained the least volume of air at all levels of pressure. Their volumes at a given pressure were about one-half as great as that of lungs of newborn infants without hyaline membranes. The average weight of the infants with hyaline membrane disease was 2.29 kg.; of the stillborn infants, 2.16 kg., and of the newborn infants, 1.82 kg. The average lung weights were 46, 40, and 38 gm.

From these measurements the magnitude of differences in functional residual capacity (F. R. C.) and pulmonary compliance were estimated. The average F. R. C. of newborn infants, assuming a value for the end-expiratory interpleural pressure similar to that in healthy seated adults, was about 65 ml. Pulmonary compliance was 3.3 ml. of $H_2O$ per centimeter. The values for infants with hyaline membrane disease were about 17 ml. and 0.8 ml. of $H_2O$ per centimeter, respectively. These estimates compare closely with direct measurements of living infants; there are no available measurements of F. R. C. in infants ultimately proven to have had hyaline membrane disease.

### 60. Rates of Thyroxine Metabolism in Children

HESKEL M. HADDAD* and J. E. RALL,* National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.
Introduced by Lawson Wilkins

Radioactive thyroxine ($T_4$) turnover rates were studied in 17 children (aged 3 to 11 years) who were euthyroid and mentally retarded but showed no evidence of a metabolic disturbance. Height and osseous development were average for chronological ages. $I^{131}$-labeled $T_4$ was administered intravenously; serum samples were collected at 10 minutes and at daily intervals thereafter for 14 days. Urine and stool excretion data were obtained in two cases. A two-compartment-system model was used for the calculation of plasma $T_4$, total extrathyroidal $T_4$ (TETT), and $T_4$ degradation and fractional turnover rates.

In addition, serum $T_4$-binding protein (TBP) was assayed in six children.

| | Child Mean | Adult Mean |
|---|---|---|
| $T_4$ $t_{1/2}$ (half-life), days | 4.95 | 6.7 |
| Plasma $T_4$ (Compartment 1), μg. | 46.2 | |
| TETT (Compartment 1+2), μg. | 129.5 | 548.0 |
| Fractional turnover rate, %/day | 13.9 | 10.5 |
| Degradation rate, μg/day | 19.1 | 57 |
| PBI, μg/100 ml. | 6.09 | 6.10 |
| TBP, μg/100 ml. | 0.248 | 0.220 |

While children maintain serum PBI and TBP at levels not significantly variant from those of euthyroid adults, they tend to utilize significantly smaller quantities of $T_4$ daily at a markedly higher rate of turnover than in adults.

### 61. Therapy of Acute Bacterial Meningitis: A Controlled Antibiotic Study

ROBERT J. HAGGERTY* and MOHSEN ZIAI,* Harvard
Introduced by Charles A. Janeway

In spite of a dramatic reduction of the mortality rate from acute bacterial meningitis since the introduction of antimicrobial therapy, this illness of infancy and childhood remains a major cause of death. In 1956, meningitis (nonmeningococcal) was recorded as the cause of death in 502 children 1 through 14 years of age in the United States, ranking it as the sixth commonest cause of death in that age group. In addition, some two to three times this number have permanent neurological residuals from this illness.

One of the major differences of opinion concerning therapy of patients with meningitis involves the question of possible harm from the simultaneous use of multiple antimicrobial agents. Those in favor of using multiple agents cite as arguments (1) the more prompt administration of the initial dose (since awaiting precise diagnosis from a stained smear of cerebrospinal fluid is then unnecessary), (2) the avoidance of giving the wrong antimicrobial agent when an error in interpretation of the original smear is made, and (3) the possible synergistic action of multiple agents.

Those favoring the use of a single specific agent argue that (1) the use of single agents in certain experimental animal infections has been shown to be superior to the use of certain "antagonistic"

* By invitation.

combinations of antibiotics; (2) the finding of Dowling, in a small series of pneumococcal meningitis, that the mortality rate was greater when multiple antibiotics were used than with penicillin alone; (3) the risk of developing resistant organisms, when more than one antibiotic is used, is increased.

A controlled experiment to test these different views has been in progress for over two and one-half years in this institution. Conclusions from this study may also be applicable to therapy of other bacterial infections.

Patients with a diagnosis of acute bacterial meningitis have been assigned to a single drug regimen if the day of admission was a odd day of the month (1, 3, 5, etc.) or to a multiple drug regimen if an even day of the month (2, 4, 6, etc.).

The method of random selection proved to be valid, for the patients were found to be comparable by analysis of their age, sex, duration of symptoms, and a rating scale of severity.

Indices followed have included survival; time required to sterilize the cerebrospinal fluid; time required for sugar, protein, and cells in cerebrospinal fluid to return to normal; duration of fever; incidence of acute complications, and general neurological status one month after discharge. Over 50 patients with H. influenzal meningitis, 35 with meningococcal, 20 with pneumococcal, and 17 in the "unknown" group have been studied in this controlled fashion.

If all types of bacterial meningitis are pooled, in the group given a single antibiotic there were 5 deaths among 54 patients, while in the group given multiple antibiotics there was 1 death among 67 patients. This difference is not statistically significant. Comparisons of the other indices among the different etiologic groups will be presented with a discussion of the significance of their findings in the management of patients with acute bacterial meningitis.

### 62. A Comparison of the Paper and Starch-Block Electrophoretic Methods for $A_2$-Hemoglobin

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Cornell
Introduced by Murray Davidson

In 1955, Kunkel and Wallenius reported the presence of a normal minor hemoglobin component with an electrophoretic mobility similar to E-hemoglobin. In normal persons the amount was 1.8%-3.2%, and in those heterozygous for thalassemia, 2.2%-9.5% of total normal hemoglobin. The percentage of this component, termed the $A_2$-fraction, is useful along with conventional hematologic measurements in the diagnosis of persons heterozygous for thalassemia. Kunkel, Singer and Gerald independently have used the starch block method for the preparation of hemoglobin. However, the equipment and time required by this method make it impractical for the average laboratory. The filter-paper method offers a quick and convenient method for the fractionation of hemoglobin fractions of all types.

The method of starch-block electrophoresis is that described by Kunkel and Wallenius. The paper method utilizes the Spinco electrophoretic cell, bromphenol blue for the staining of the patterns, and the Spinco Analytrol for pattern analysis.

The results of the starch method for the analysis of the $A_2$-fraction of the hemoglobin in 22 normal patients ranges from 1.6%-3.2% and in the patients known to be heterozygous for thalassemia from 3.0%-9.3%. With the paper method the results show the range for 20 normals is 5.0%-14.3% and 13.2%-27.0% for 35 known thalassemia traits. The higher values for the paper method are due to an unknown X-protein which can not, thus far, be eliminated and which migrates and stains with the $A_2$-fraction. The percentage of $A_2$ present in a few persons known to be heterozygous for thalassemia by other criteria fall within the normal range by both starch and paper methods. Therefore it can be said that only those values for $A_2$ which lie above the zone of overlap in both paper and starch methods are diagnostic for the heterozygous thalassemia state.

### 63. The Effect of Oral Salt and Urea Loading on Renal Concentrating Ability in Rats

L. LEIGHTON HILL* and M. HETRICK,*
Western Reserve
Introduced by William M. Wallace

Gamble et al. noted that rats fed large quantities of urea in their diets and allowed water ad libitum drank less water and excreted a more concentrated urine than if the urea were replaced by an osmotically equivalent quantity of inorganic salts. This suggested an economy of water referable to urea. The present experiments were undertaken to test this relationship under conditions of relative water restriction in which the kidney would be under stress to achieve a maximally concentrated urine and extrarenal factors controlling intake of water would not be operative.

Each animal was confined in a separate metabolism cage; consecutive 24-hour urines were collected, and the measurement of water and food intakes were recorded. Three groups of rats were studied. Control data were obtained on all groups on the basic diet. Alternate rats in each group had 2 mOsm. of either sodium chloride or urea

* By invitation.

added to each gram of basic diet. One group of animals received ad libitum water; the second group was limited to control intakes (9 ml/100 gm. per day) for five days. A third group was also limited to their control intakes but for a more extended period. Urines were analyzed for sodium, potassium, chloride, and urea, and osmolalities were obtained on all urines.

On ad libitum intakes the urea-loaded animals drank less water and ate more food than the salt-loaded group. As in any osmotic diuresis, the salt-loaded rats experienced a decrease in concentration during loading. There was a slight rise in concentration (800 mOsm. per kilogram) on the fifth loading day, but this was well below the control concentration of (1,200 mOsm. per kilogram). There was no significant change in the urine osmolality of the urea-loaded animals (control, 1,465 mOsm. per kilogram; fifth loading day, 1,500 mOsm. per kilogram).

In the water-restricted groups the urea-loaded animals again excreted more concentrated urines than the salt-loaded animals. The same initial reduction in urine osmolality was observed in both groups of salt-loaded animals, followed by a rise to concentrations above those obtained in the control periods. The concentration in the salt-loaded animals during the five-day loading experiment was highest on the last day, when an osmolality of 1,500 mOsm. per kilogram was obtained. In the longer experiment performed to evaluate possible adaptation, the salt-loaded rats reached a maximum of 2,750 mOsm. per kilogram. The urea-loaded animals achieved a concentration of 1,900 mOsm. per kilogram on the last day of the first water-restricted experiment and a concentration of 3,500 mOsm. per kilogram during the prolonged experiment. The intake of food decreased in all animals but to a greater extent in those receiving the diet to which salt had been added. All animals became dehydrated, the salt-loaded ones losing an average of 6.9% of body weight and the urea-loaded animals 4.5% on the first day of loading. In the prolonged experiment the urea-loaded animals began to gain weight on the 13th day of loading; the salt-loaded group continued to lose weight until the end of the experiment.

Thus, it seems apparent that under conditions of relative water restriction, eliminating the factors of thirst and providing a stress to conserve water, oral loads of urea result in a more concentrated urine than when equal osmolar loads of sodium chloride are given. During prolonged loading there is evidence of adaptation in all animals, but this is more marked in the urea-loaded group.

### 64. Recurrent Pyelonephritis Without Urinary Tract Abnormality

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Cincinnati

Recurrent urinary tract infections of sufficient severity to be challenging and frustrating problems have been observed and followed in six children over periods of one to four years. All were girls varying in age from 3½ to 8½ years. Their course has been characterized by recurrent bacteriuria with high urine colony counts, with or without accompanying pyuria, fever, and symptoms referable to the urinary tract. Adequate courses of appropriate antibiotics result in disappearance of symptoms and sterilization of the urine, but infection, usually with another organism, has recurred within a period of two or more weeks. In one child six such recurrences were observed in a six-month period. Roentgen and urological diagnostic procedures, many of them repeated several times, failed to reveal an obstructive lesion or evidence of abnormal physiology of the urinary tract. The possibility that susceptibility to infection was on the basis of an intrinsic renal abnormality of the dysplastic type, as described by Marshall, was not supported by renal biopsy in three of the children.

Since the infections could be eliminated by intensive antibiotic therapy, the problem was that of prevention of reinfection. To accomplish this the following regimen of prophylaxis has been employed during the past 18 months. First infection was eradicated by antibiotic agents. Immediately after cessation of this therapy, methenamine mandelate, 2 gm. per square meter per day, was started and given continuously. Urine pH has been tested daily by the parents by means of indicator papers and acidifying agents given as necessary to maintain the value at 5.5 or below.

The results have been encouraging. After one to three years of recurrent disease, all six patients have been free of infection for periods of 6 to 17 months while on this regimen. Cultures obtained by renal biopsy have been sterile and the tissue histologically normal. No untoward effects of prolonged therapy with methenamine mandelate have been observed.

### 65. The Distribution of Water Loss of Hypernatremic Dehydration

MALCOLM HOLLIDAY and JAMES PITCAVAGE,*
University of Pittsburgh

Studies on the composition of deficits associated with hypernatremic dehydration due to water deprivation and salt loading were compared to similar studies of hypernatremic dehydration due to water deprivation and urea loading. In several studies

* By invitation.

the two regimens were applied to rats weighing 200-400 gm. from five to seven days. When smaller animals were used, all animals in both groups died by the fifth day. Mortality was less in the succeeding studies where larger rats were used but was equal for the two experimental regimens, establishing equal severity of salt and urea loading as a cause of death.

The weight loss among survivors of the salt- and urea-loaded groups was also comparable, 33%-34% of the initial weight. The distribution of the weight loss was determined by comparing carcass analyses of the depleted animals to those of comparable control animals. The weight loss was distributed as follows:

| | Total | Fat | Nonfat Solids | Water | ECW | ICW |
|---|---|---|---|---|---|---|
| Salt | 115 | 12 | 14 | 77 | 15 | 62 |
| Urea | 120 | 10 | 14 | 80 | 23 | 57 |

From these data the loss of water was seen to greatly exceed the loss of nonfat solids, describing a severe dehydration. From the changes in volume of distribution of chloride, much of the dehydration was intracellular. Parallel changes in extracellular and intracellular volume were observed in a sample of muscle. Serum sodium concentration correlated with concentration of potassium in muscle water and inversely with the intracellular water of both muscle and carcass. The increase in serum sodium concentration was less marked in the urea-loaded group, but a nearly comparable increase in potassium concentration of muscle water was observed. The data confirm the general concept for the distribution and effects of hypernatremic dehydration. From the data, it is inferred that the degree of intracellular dehydration is a more important determinant of mortality than the degree of hypernatremia. It was noted that most of the salt given in this experiment was excreted, and salt loading with dehydration did not lead to over expansion of extracellular volume but rather some loss of extracellular fluid.

### 66. The Diurnal Serum Lipid Levels of Children With and Without Cystic Fibrosis of the Pancreas

NANCY N. HUANG * and PETER T. KUO,*
St. Christopher's and Hospital of
University of Pennsylvania
Introduced by Robert H. High

A decrease in absorption of fat has been frequently demonstrated in children with cystic fibrosis of pancreas. This naturally imposed restriction of fat, which is controllable in some measure by pancreatic extract, offers a unique condition for the study of the effect of dietary fats upon the serum lipid concentration and of its association with atherosclerosis.

Diurnal serum lipid variations of nine children with cystic fibrosis of the pancreas on mixed diets supplemented with predigested proteins and carbohydrates and similar studies in seven normal controls were made before and after Viokase (whole-pancreas powder) administration. Serum triglyceride, phospholipid, and cholesterol levels were determined at 4-hour intervals for 24 hours.

In none of the children were any consistent diurnal serum cholesterol or phospholipid patterns observed. In the normal and diseased groups the mean values of the serum cholesterol concentrations were 175 and 125 mg. %, respectively, and the phospholipid values were 180 and 165 mg. %, respectively.

In each of the nine patients with cystic fibrosis of pancreas the diurnal serum triglyceride curve remained flat following meals. The curves dipped slightly after midnight then rose slightly with prolonged fasting. After administration of Viokase, the serum triglyceride curves of six patients with cystic fibrosis of pancreas changed toward those of normal children, showing significant postprandial rises and sharp dips to the respective fasting levels before breakfast. Viokase administration did not alter the diurnal serum triglyceride curves of the normal controls. Despite the chronic decrease in absorption of fat, the mean fasting serum triglyceride concentration of the patients with cystic fibrosis of pancreas was the same as that of the normal children.

The data indicate that cystic fibrosis of the pancreas primarily affects the diurnal serum triglyceride levels of the patient. It is interesting to note that decreased atherosclerosis has recently been observed in children dying of this disease.

### 67. Effects of Human Growth Hormone and of Modified Bovine Growth Hormone

JOHN J. HUTCHINGS,* ROBERTO F. ESCAMILLA,*
WILLIAM C. DEAMER, and C. H. LI,*
University of California Medical Center
Introduced by Henry B. Bruyn, M.D.

Human growth hormone prepared by the Li method from pituitaries removed at autopsy was administered to a 13-year-old girl with pituitary infantilism. Metabolic balance studies demonstrated retention of nitrogen, potassium, sodium, and phosphorus. Calcium balance was negative initially but became positive with long-term administration. Effective nitrogen retention was accompanied by a drop in the BUN. The preparation was administered for a year with a fourfold increase in rate of linear growth without disproportionate skeletal maturation. Chemical observations and data on gonadotropic and adrenal secretion were made periodically.

After this initial study human growth hormone was given for short periods of time to two addi-

* By invitation.

tional children with pituitary infantilism and to two children with gonadal dysgenesis. The effect was found to be similar, although there were indications of individual variation in response and the children with gonadal dysgenesis did not respond to the same degree as that seen in pituitary infantilism.

Since the past use of bovine growth hormone has been disappointing, producing only equivocal metabolic changes and failing to produce satisfactory growth, an attempt has been made to modify this preparation in order to increase its effectiveness. The bovine growth hormone has been subjected to chymotrypsin digestion, yielding a small molecular core that retains its growth-promotion activity and yet is free of sensitization effect. This preparation was given both for short-term metabolic studies in four of these children and long-term administration in two of the children. The metabolic effects were less profound than those with the human preparation; the reproducibility of these effects was less reliable, and response in linear growth has been less marked.

Diabetogenic effect and fluid retention were observed during short periods of administration of high doses of human growth hormone, but these effects were transient and seem related to dose level. Similar toxicity was not seen in the modified beef preparation, and there was no indication of the marked allergic response which had occurred with the use of unmodified bovine growth hormone.

## Discussion

DR. ROBERT BLIZZARD, Columbus, Ohio: I would like to ask if this patient had glomerular filtration rate and renal plasma flow measurements. I also would like to know what happens to nitrogen retention in a normal individual administered human growth hormone. In addition, did you do a water-loading test in this patient before and after therapy with human growth hormone?

DR. DAVID W. SMITH, Madison, Wis.: With regard to the patient who received long-term pituitary growth hormone administration, would you please give the evidence for hypopituitarism? This is a difficult diagnosis to substantiate during childhood unless one can demonstrate evidence of a lack of pituitary trophic hormones beyond pituitary growth hormone.

DR. HENRY L. BARNETT, New York: I'd be interested in knowing about the diet the patient received during the metabolic balance periods, particularly in regard to the caloric intake.

DR. JOHN F. CRIGLER JR., Boston: I'd like to ask how long nitrogen retention, as measured by balance, persists with the administration of growth hormone. What was the nitrogen retention in terms of nitrogen intake?

Thirdly, how do you interpret the alterations you described in the glucose tolerance tests?

DR. IRVING SCHULMAN, Chicago: Dr. Hutchings, was there any evidence that red cell and hemoglobin production were stimulated during long-term hormone administration?

DR. MELVIN GRUMBACH, New York: Dr. Hutchings, could you tell us a little bit about the bone maturation in this child. Was there any change in the rate of maturation?

DR. LYTT I. GARDNER, Syracuse, N.Y.: I'd be curious to know if you made serial measurements of serum inorganic phosphorus and if there were any changes in that?

DR. CLAUDE J. MIGEON, Baltimore: It is of interest that the gonadotropin assay became positive under growth hormone therapy. Do you know if your growth hormone preparation was, to a certain degree, contaminated with pituitary gonadotropins? If it was, the increased rate of statural growth of the patient could be related to the production of sex hormones stimulated by the administration of human pituitary gonadotropins as well as being related to the growth hormone therapy.

DR. JUDSON J. VAN WYK, Chapel Hill, N.C.: Dr. Hutchings, was this child clinically hypothyroid and did this excellent growth take place in the absence of thyroid substitution therapy?

DR. JOHN D. BAILEY, Toronto: I would like to ask if insulin sensitivity tests had been done and if there was any change in insulin sensitivity while the patient was on the human growth hormone.

DR. HUTCHINGS: Dr. Blizzard, we did not do specific renal function studies or water-loading tests on this patient. We have been surprised by the number of patients with suspected hypopituitarism who have a high BUN. Most of these patients have had urea or creatinine clearances and many of them have had intravenous pyelograms which were normal. Animal work shows that growth hormone does increase renal blood flow and glomerular filtration, and we hope to do this kind of study in the human. I would be most interested to hear about the experience of others concerning the BUN in patients with suspected hypopituitarism.

We do not yet know whether nitrogen is retained in a normal person when human growth hormone is administered, but hope to study this soon.

Dr. Smith, we are aware of the difficulty in establishing the diagnosis of hypopituitarism in childhood, since no test for growth hormone is widely available at present. Our patient with long-term hormone administration had a history in keeping with encephalitis at the age of 6 months, followed by growth cessation. When we first saw her at 9 years of age there was evidence of a

neurological residual in addition to her growth retardation. Her height and bone age were those of a 4½-year-old. Her urinary excretion of 17-ketosteroids and 17-hydroxycorticoids was low for age and probably for body size also. Thyroid function study results were normal, except for her $I^{131}$ uptake which was borderline (17%) and showed a striking response to the administration of TSH. She had no symptoms of hypothyroidism. She was put on sodium L-thyroxine and continued on it for 2½ years. During that time her bone age increased from 4½ to 9 years, but her growth rate remained at a level below that of the third percentile. We discontinued thyroid therapy six weeks before our study began. Thyroid and adrenal function tests, as demonstrated in our report, were in keeping with the diagnosis of hypopituitarism.

Dr. Barnett, all of the these children were on exactly the same diet every day during the balance studies. There were no substitutions or exchanges. The patient on the long-term study was on a diet of 1,600 calories containing 70 gm. of protein, 70 gm. of fat, and 172 gm. of carbohydrate. Sodium was 1,158 mg., potassium 2,728 mg., calcium 1,164, and phosphorus 1,331 mg. We were extraordinarily lucky not to have dietary rejections, except with one patient. The other four have managed to consume their entire intake for a long period of time. In general, we have kept the total intake low so that they will continue to tolerate it.

Dr. Crigler, in our first patient, nitrogen retention seemed to persist only for the 24 hours following hormone cessation, as with another patient. However, in the one boy whose balances I demonstrated, it did persist for 48 to 72 hours. Workers using the Raben preparation have also found this persistence for several days. The variation, apparently in individual response, has made our interpretation of the data using the modified beef preparation more difficult because in some of these studies we had retention following the period of administration which was equal to or exceeded that during the period when the patient actually received the hormone.

In our patient who has been studied most intensively, nitrogen intake was 11 gm. and hormone administration caused a retention between 1 and 2.5 gm. Our other patients have been comparable, although the patient with gonadal dysgenesis was less responsive, as indicated on the slide.

Interpretation of the alterations in glucose tolerance test is difficult in that growth hormone seems to affect carbohydrate metabolism on many levels. Its effect, when given to the intact organism, could be called—for want of a better word—"diabetogenic." In the pituitary-deficient individual this would not cause difficulty if one could supply a dosage that might bring the carbohydrate metabolism only into physiological balance. In our patient we exceeded that dosage and converted a low glucose tolerance curve to a diabetic one, but the change was reversible, as demonstrated. On the other hand, two separate groups of workers have had complications when human growth hormone was administered to hypophysectomized diabetic patients, with production of marked hyperglycemia and severe acidosis. Certainly anyone who administers this preparation to diabetics should exercise great care. The possibility exists that we might ultimately find a fraction of the hormone which would have the nitrogen-retaining effect dissociated from the carbohydrate changes. There is some evidence from our work with the modified beef preparation that this potentiality exists.

Dr. Schulman, we noticed no significant change in hemoglobin, red count, or PCV in these patients, though I am aware that Dr. Raben has reported a slight increase in hemoglobin. We did not do reticulocyte counts or more detailed studies.

Dr. Grumbach, during one year's period of hormone administration less than one year's maturation occurred in bone age. We were, of course, most pleased with this, since this has been the limiting factor in so many preparations given to stimulate growth in dwarfed children.

Dr. Gardner, we found no significant and consistent change in serum inorganic phosphorus although we made frequent serial measurements of this substance. This was in contrast to reports on animal work and to recent reports of workers using the Raben preparation.

Dr. Migeon, we were also interested in the changes in gonadotropin assay. The original growth hormone preparation was assayed by Dr. Li, using fairly specific tests, i.e., follicle-stimulating hormone activity by an augmentation test using increase in ovarian weight in the immature rat and interstitial cell-stimulating hormone by increase in the ventral prostate in the immature, hypophysectomized male rat. By these more definitive tests the human growth hormone seems free of gonadotropic effect.

When we began to check the patient we used the routine hospital test, which is a modification of the determination using increase in uterine weight in the immature white Swiss mouse as an end-point. As you have seen, by this less specific test our patient began to show a positive assay. I then assayed a small amount of the growth hormone preparation with our routine hospital test and it was positive. Recently, Dr. Li demonstrated that growth hormone given to a hypophysectomized, thyroidectomized, adrenalectomized, and gonadectomized animal still causes an increase in uterine weight. Presumably this means that

growth hormone has a direct effect on uterine growth and this may be the explanation for the positive assays on our patient. However, the possibility does exist that in given growth hormone we are stimulating the pituitary itself to produce endogenous gonadotropin.

I do not, however, think the increased statural growth of the patient is related to sex hormone production since the patient has had no evidence of any sexual maturation. Her bone age is 9½ years, and I think if adolescence had been initiated we would have had acceleration of the bone age and perhaps some evidence of adenarche, which would have been reflected in ketosteroid excretion as well as bodily changes. None of these have occurred.

Dr. Van Wyk, the child has never been clinically hypothyroid, and earlier thyroid therapy gave no increase in linear growth. The growth that we have reported with our preparation occurred in the absence of any other therapy.

Dr. Bailey, this patient does show insulin sensitivity, but we have not attempted to use this test serially as we have had difficulty using it on patients with hypopituitarism without inducing a state of clinical hypoglycemia.

### 68. The Influence of Rocking on the Respiratory Performance and Initial Weight Loss of Premature Infants

JERRY C. JACOBS,* DANIEL S. ROWE,* and WILLIAM A. SILVERMAN, Columbia

Seventy-two infants weighing 1,960 to 2,490 gm. were entered in a study to ascertain the influences of rocking on respiratory performance as judged by rate and retraction score and upon initial weight loss. The population was subdivided into two weight groups. Within one hour of birth the infant was weighed in the nursery and a sealed envelope was selected from a sequence which had been predetermined from a table of random permutations for each birth-weight group. Infants selected to be rocked were placed supine on the Isolette Rocker, which was set at a 20-degree angle and rocked 20 cycles per minute for 12 hours. Control infants were placed supine in a standard Isolette. No oxygen was used in either group except to relieve cyanosis. The rocker was operated by an air compressor, not by oxygen, as our preliminary studies revealed that operation of this machine by oxygen raised the oxygen level of the Isolette to 22%-24%. Infants were thirsted during the study period and received a predetermined combination of antibiotics only on indication. The infants were evaluated once every 6 hours for the first 36 hours of life. At these times the respiratory rate and retraction score were noted according to the criteria previously established at this hospital. Each infant was examined during the study period by one of us. The four study groups were comparable with respect to birth weight, sex, race, serology and blood type of mother, birth rank, Apgar score, and mode of delivery.

No statistically significant difference in respiratory rate or weight loss could be discerned between those rocked and the controls. In both weight groups the respiratory rate was slower and the weight loss greater in those who were rocked (see accompanying chart), but these differences could be expected to occur easily by chance. So few babies had any retractions that this observation did not lend itself to statistical analysis, but this evidence of distress seemed equal in the two groups. Three babies died in the first week of life. Two of these had been rocked; one died with hyaline membrane disease; postmortem examination on the other revealed chronic pneumonia and fatty metamorphosis of the liver. The control infant was thought clinically to have myocarditis, but at postmortem examination the findings were consistent with anoxia; pulmonary edema and subarachnoid hemorrhage without evidence of damage to the underlying brain were also present. The latter two babies (one rocked and one not) had received chloramphenicol (Chloromycetin) in dosage (50 mg. per kilogram q. 12 h.) larger than that now recommended; the indication for antibiotic therapy was premature rupture of the maternal membranes. Eight infants and their weight-matched controls were eliminated from certain parts of the analysis because inadequate data were obtained.

One of the rocked infants was noted at five days to have evidence of impaired circulation in the right leg, with an absent right femoral pulse and a pale cold extremity. This was treated with local measures, and gradually all of these signs subsided. When seen at follow-up visits after discharge this infant appeared normal.

Under the conditions of this study rocking from age 1 hour to age 12 hours had no significant effect

| | 1,960-2,249 Gm. | | 2,250-2,490 Gm. | |
|---|---|---|---|---|
| | Rocked | Controls | Rocked | Controls |
| Infants, No. | (8) | (8) | (21) | (21) |
| Mean respirations/min. | 50 | 54 | 47 | 48 |
| Standard deviation | 17 | 13 | 8 | 10 |
| Infants, No. | (7) | (7) | (20) | (20) |
| Mean weight loss, gm. | 130 | 119 | 146 | 135 |
| Standard deviation | 22 | 46 | 54 | 50 |

* By invitation.

on the respiratory performance or initial weight loss of a group of infants in this weight category. No adverse effects of rocking were noted. This study was not designed to evaluate the effects of rocking infants at delivery or the use of the rocker as a resuscitator in apneic infants. Similarly designed studies should be carried out to evaluate these functions of rocking.

### 69. Observations on an Infant with the Congenital Nephrotic Syndrome

MORAD JACOBSON,* HARVEY BUCHMAN,* ELIZABETH KAISER,* ARTHUR ROSENBLUM,* MARY ZACHARIAS,* TINY SHARP,* and JACK METCOFF, Michael Reese Hospital, Chicago

The congenital nephrotic syndrome is extremely rare, and hence physiologic and renal biopsy data are limited. A 3-month-old boy of Scandanavian-Scotch descent, one of three siblings having the nephrotic syndrome at birth, was repeatedly studied for six months. Discrete renal functions; electrolyte and nitrogen balances; metabolism of $I^{131}$-albumin and $I^{131}$-polyvinylpyrrolidone (PVP); plasma and urine protein patterns (including those of parents), amino acid excretions, studies of response to a diuretic (chlorothiazide) and N-retaining steriod (17 hydroxynorandrosterone); electrolyte composition and some intermediary metabolites of muscle obtained by biopsy, and electron microscopy of a renal biopsy specimen have been completed.

The data highlight the peculiar nature of the congenital nephrotic syndrome. Virtually no growth has been noted over the six-month period. Initally, GFR was normal although the filtration fraction was reduced. RPF/Tm and GFR/Tm were within normal limits. Despite edema, Na and K metabolism were not abnormal initially, but later in the course of the disease, the typical nephrotic pattern was observed. Proteinuria averaged 1-2 gm/24 hours, but $t\frac{1}{2}$ of injected tagged albumin (and PVP) were extremely short, approximately 18 hours.

Increased synthesis and, especially, degradation of albumin were noted. Neither cholesterol nor $\beta$-lipoproteins were increased. Neither chlorothiazide nor nitrogen-retaining steroid altered edema or N balance. Muscle showed decreased cellular content of K and total and organic P; electron micrographs of renal biopsy material revealed absent epithelial-cell foot processes of the glomerular capillaries, the characteristic lesion of the nephrotic syndrome.

Simultaneous occurrence of a metabolic defect (increased protein catabolism) little or no gross lipid disorder, unusual cellular CHO metabolite and electrolyte concentrations, and a definitive renal lesion (absent foot processes) in the congenital form of the nephrotic syndrome seems of particular interest.

### 70. The Effect of Certain Substances on Bilirubin Levels and Occurrence of Kernicterus in Jaundiced Rats

LOIS JOHNSON,* ESPERANZA FIGUEROA,* MARIA LUISA GARCIA,* and HAROLD NEWMARK,* State University of New York, Brooklyn
Introduced by Richard Day

Previous work in this laboratory has shown that the Gunn strain of genetically jaundiced rats is useful for studying substances which might influence bilirubin levels and kernicterus. Since bilirubin is bound to protein, other substances might compete for binding sites, an idea studied by Odell in in vitro observations.

1. *Sodium Salicylate* (suggested by Odell).—This lowered the serum bilirubin of five adult rats by 49% to 62%. No kernicterus developed. Thirteen newborn rats responded with a similar fall of bilirubin, and all developed kernicterus. Twelve of the thirteen died. The expected kernicterus rate is 58%, and mortality from kernicterus is 35% (based on observations of 181 untreated rats).

2. *Sodium Glucuronate.*—Holt et al. found and Schmid et al. failed to find a lowering of bilirubin levels in human infants. Odell (personal communication) found no interference with protein binding of bilirubin.

Intravenous sodium glucuronate in 15 adult rats lowered bilirubin from 6% to 48%. No kernicterus resulted. Of 25 newborn rats given injections, 19 (76%) developed kernicterus; 11 of these (44%) died.

3. *Fat Emulsion.*—Fat emulsions injected intravenously are known to be converted rapidly into fatty acids, which are bound to albumin. In eight adult rats given Lipomul intravenously, bilirubin levels fell from 34% to 88%. As the Lipomul was cleared from the blood stream, the bilirubin levels returned to base line. No newborn rats were given injections with Lipomul.

4. *Human Albumin.*—The degree of saturation of intravascular protein-binding sites plays a role in the distribution of bilirubin in the body. Therefore, the protein levels of maturing rats and the effect of injections of albumin are of interest. In five fetuses about two days before term total serum protein was 1.9 gm/100 ml. In 11 rats at 7 days the level was 2.6 gm/100 ml.; in 7 rats at 15 days it was 4.1 gm., and in 6 rats at 20 days, it was 4.6 gm. The adult level (20 rats) is 6.5 gm. Since rats which develop kernicterus do so usually by 15 days of age and always by 21

* By invitation.

days, it is possible that the rise in protein level offers some protection against kernicterus.

Forty-six newborn rats were given 0.5 to 1.0 gm. of human albumin per kilogram per day intraperitoneally from the age of 2 to 5 days until age 21 unless death supervened. Of these, 11 developed kernicterus (23.9%) and 7 died (15.2%). Bilirubin levels were higher than in control animals. At 14-16 days the bilirubin averaged 14.1 mg. %, as compared with 41 controls, which at this age had an average level of 11.5 ±2.58 ($P<0.01$). Five animals given injections of albumin survived and escaped kernicterus despite bilirubin levels of 22.6, 23.0, 23.7, 20.4, and 20.1 mg/100 ml. One survived a bilirubin level of 24.3 mg. but developed kernicterus. The five highest serum bilirubin levels survived by untreated animals have been from 15.0 to 19.9 mg/100 ml., and all five of these animals developed kernicterus. These findings suggest that the incidence of the disease had been altered ($P<0.01$). An alternative explanation would be a spontaneous change in the rat colony. The seeming benefit from the human albumin therefore needs confirmation with simultaneous controls.

### 71. Studies of Red Cell Survival in Early Infancy

EUGENE KAPLAN, Sinai Hospital, Baltimore

The survival of erythrocytes of newborn infants has been studied in an attempt to determine the role of hemolysis in the development of neonatal physiologic anemia. Red cell half-life ($\tau\frac{1}{2}$) was determined by autotransfusion or heterotransfusion (into normal adults) of $Cr^{51}$-tagged erythrocytes from 16 premature and 14 full-term infants ranging in age from 0 to 120 days. In addition, $\tau\frac{1}{2}$ was determined for seven specimens of blood taken from placental veins.

Red cell survival was at normal adult levels ($\tau\frac{1}{2}$ 25-35 days) for each of five infants studied during the first week of life. Survival was shortened for 14 of 19 infants ($\tau\frac{1}{2}$ 12-21 days), between age 3 and 9 weeks and in 2 of 6 infants between 10 and 16 weeks ($\tau\frac{1}{2}$ 11 and 19 days). No differences were found in this small group of infants between premature and full-term infants of the same age. In three of seven samples of placental blood, $\tau\frac{1}{2}$ was shortened to 16, 19, and 20 days.

Radiochromium uptake and elution by *intact* erythrocytes was not affected by their fetal hemoglobin concentration. No differences were found where comparisons could be made of the results as measured by autotransfusion and heterotransfusion.

The normal erythrocyte survival of the first week of life suggests that hemolysis does not play an important role in the development of physiologic anemia of the newborn. The reduced survival of the second and third months is consistent with the concept that reduced erythropoietic activity of the first weeks of life results in a disproportion of older erythrocytes in the blood at 2 and 3 months of age, with a mean erythrocyte survival less than that of a normal red cell population. The contradictory behavior of placental red blood cells remains to be explained.

#### Discussion

DR. IRVING SCHULMAN, Chicago: In performing red cell life span studies with $Cr^{51}$ one may express the data as counts of radioactivity per milliliter of packed erythrocytes or as counts per ml. of whole blood. If one employs the former method one may obtain information relating to degrees of hematopoietic activity in the babies. If, as is commonly believed, the first several weeks of postnatal life are characterized by markedly reduced hematopoiesis, the curve of radioactivity in counts per milliliter of whole blood would be relatively flat. When erythropoiesis resumes a sharp break in the curve would become evident. I wonder if Dr. Kaplan has calculated his data in this way and could give us some information concerning when blood formation resumes in the neonatal period?

DR. WILLIAM KRIVIT, Minneapolis: As measured by the $Cr^{51}$ method, the abnormal red cell survival in infants has been attributed to the elution phenomenon. Our data to be published indicate that elution rate of $Cr^{51}$ from infants' red cells in vitro is not different from that of adult red cells. I would like to hear your data about this elution phenomenon.

The survival of normal adult blood transfused ing normal newborns was also studied by the $Cr^{51}$ method. Surprisingly, the $\tau\frac{1}{2}$ of $Cr^{51}$ was only 16 days. Therefore, I have postulated that there is an extracorpuscular effect in the normal infant. The presence of such a "normal" extracorpuscular process, if confirmed, would suggest that only autotransfusion studies will provide the desired analysis of survival of normal newborn red cells.

DR. JOSEPH DANCIS, New York: We have approached the problem of the life span of the red blood cell in the premature infant, using $N^{15}$ to tag the hemoglobin. There is a basic difference in tagging by this method and that of $Cr^{51}$, but the results agree very well with those of Dr. Kaplan. With chromate a mixed population of red cells of different ages are tagged, yielding results that can be interpreted as "half-life." With $N^{15}$, the tagged cells are of the same age so that disappearance of the label indicates "life-span." The $N^{15}$ method has certain theoretical advantages over chromate but is far more cumbersome. As a result we have data on the life span of the red cell in

only two premature infants. The life span of half the cells approximated 110 days, which is very similar to the results in adult subjects. The $N^{15}$ curve also indicated that new cell production began to exceed the rate of destruction at about 50-60 days of life.

DR. AARON G. MEISLIN, New York: Was there any difference in red cell survival noted between infants born of homospecific pregnancies and those of heterospecific pregnancies?

DR. KAPLAN: The data expressed as counts per second per milliliter of red cells fail to reveal the resumption of erythropoiesis by any noticeable dip in the counting activity of serial samples. This is not surprising in view of the small increment of new erythrocytes expected within the brief period of our observation of any single infant.

Insofar as our studies can be compared, there has been no indication of any significant difference in the survival rates of autologous or heterologous transfused erythrocytes of normal newborn infants. We have found no differences in the rate of chromium elution from intact erythrocytes of varying fetal hemoglobin concentration.

Concerning the effect of heterospecific pregnancy on neonatal red cell survival, with only few exceptions in these reported studies the infants were of homospecific pregnancies or were blood group O and their mothers blood group A or B. Two normal full-term infants, one group B and the other group A, born to group O mothers, were studied in their first 5 days. The erythrocyte survival times observed were $\tau\frac{1}{2}$ 29 days and 33 days, auto- and heterotransfusions respectively. In addition, two infants with mild ABO hemolytic disease studied on the first day yielded shorter survival of 22 days, one following autotransfusion and the other following heterotransfusion.

The $N^{15}$ glycine studies of Dr. Dancis are most important. They avoid the serious mechanical limitations inherent in transfusion techniques, and establish essentially normal values for the survival of erythrocytes in the premature infant. Nevertheless, it must be emphasized that information from these studies pertains only to postnatally produced erythrocytes, and not to those exposed to intrauterine environment.

### 72. The Renal Response of Infants to Dehydration and Acidosis Induced by Diarrhea and by Artificial Means

S. A. KAPLAN, A. M. YUCEOGLU,* and J. STRAUSS,*
State University of New York, Brooklyn

Studies were made in which the effects of disease on the renal powers of conservation of water and of base were measured in infants hospitalized for severe diarrhea with dehydration. A comparison was made of the renal response to dehydration and acidosis due to diarrhea and of the renal response of infants subjected to artificially induced acidosis and dehydration.

Nine infants with diarrhea, estimated to have lost over 10% of their body weight as fluid, were catheterized immediately on admission to the hospital prior to therapy with intravenous fluids. The specimens of urine obtained were found to have concentrations of total solutes which varied from 386 to 770 mOsm. per liter. Since infants of this age can normally concentrate their urine to more than 1,200 mOsm. per liter (Pratt et al.: *Pediatrics* 1:181, 1948) it seems that in diarrheal dehydration there is interference with the normal mechanism of water conservation in spite of the presence of maximal stimuli. In seven of the patients the serum sodium concentration was greater than 160 mOsm. per liter. No correlation was observed between the concentration of serum sodium and urine osmolarity. The concentration of urea in the serum, which was elevated in all patients studied, likewise was not correlated with urine osmolarity. One infant whose serum sodium concentration was 180.0 mEq. per liter voided urine containing only 485 mOsm. per liter. Another, whose blood urea nitrogen concentration was 142 mg/100 ml. voided urine containing only 495 mOsm. per liter.

Ammonia production and titratable acidity in the urine were measured in the first timed sample of urine after admission and were found to be very low. Since the volume of urine formed at this time is small, it was decided to defer these measurements of base conservation for 12 or 24 hours, when acidosis was still present ($CO_2$ content less than 15 mEq. per liter) but urine flow exceeded 0.5 ml. per minute per square meter. Ammonia production per square meter in 10 patients varied from 12.5 to 77.8 mEq. per 24 hours and titratable acid from 4 to 30.2 mEq. per 24 hours.

Six normal infants less than 1 year of age were then tested for their ability to conserve base. Each subject received 6 gm. ammonium chloride per square meter of body surface in four doses per day while receiving ordinary diets. Administration of the acid-producing salt was continued until acidosis was induced as evidenced by a significant fall in the plasma bicarbonate content (usually three to four days). Urine was collected over the last day on which ammonium chloride was administered.

In these normal children subjected to acidosis, measurement of ammonia production and titratable acidity showed a response not significantly different from that of the children with diarrhea (ammonia production, 10.0-49.6 mEq. per square meter per 24 hours, and titratable acidity 14.6 to 36.0 mEq. per square meter per 24 hours). Compared with adults, however, who can conserve at least four

* By invitation.

times as much base, the response of both normal children and those with diarrhea to acidosis is poor indeed.

Our studies show, therefore, that infants with dehydration and diarrhea conserve water poorly in spite of maximal stimuli and do not produce urine which is as hypertonic as that of normal infants subjected to dehydration. Base conservation is also poor in these children by adult standards but is no worse than that of normal children of the same age subjected to artificially induced acidosis. The mechanism of interference with normal water conservation in infants with diarrhea is not clear, but possible causes are potassium deficiency and the toxic products of infective organisms. Since urine flow and glomerular filtration rate are very low in these infants, it appears that the kidneys conserve water by reducing the glomerular filtration rate rather than by reabsorption of water. This serves to explain why the concentration of blood urea nitrogen rises so rapidly in these infants.

### 73. Immunization of Children with a Live Attenuated Measles Virus

SAMUEL L. KATZ * and JOHN F. ENDERS,* Harvard
Introduced by Sidney Kibrick

A study has been conducted to determine the clinical and immunological responses of susceptible children to inoculation with a live strain of measles virus characterized by attenuated virulence for monkeys. Experiments in monkeys confirmed and extended earlier results showing that the attenuated virus produced no signs of disease, failed to enter the blood stream in detectable amounts, caused no recognizable changes in the central nervous system, but did produce antibody responses (both complement fixation and virus neutralization) in concentrations comparable to those found in animals recovering from infection with virus in the virulent form. Other preliminary studies showed that virus activity could be stabilized in Medium 199 by the addition of human serum albumin. A "vaccine" consisting of the attenuated virus in this medium was prepared and safety-tested in monkeys and measles-immune adult human volunteers.

"Vaccine" was then administered subcutaneously to 11 susceptible children. Rash or fever or both occurred in all after an incubation period of about 6-10 days. Virus was not recovered from the blood or throats of these children. The infection caused little or no discomfort, and the children continued with their accustomed activity throughout. Susceptible children in contact with those inoculated failed to become infected. Complement-fixing and neutralizing antibodies developed in the inoculated children thereafter by the 22d day in titers comparable to those encountered in persons convalescent from natural measles.

Subsequently, small trials of the same vaccine preparation have been made in collaboration with Dr. C. Henry Kempe. In addition to three susceptible children given inoculations subcutaneously, five others were given inoculations intradermally. Essentially the same reactions followed irrespective of the route. Again the discomfort was minimal. Antibody responses of the magnitude obtained in the first trial were recorded in all tested, with a single exception. In the latter, antibody was demonstrated before vaccination. Two persons vaccinated percutaneously failed to manifest a clinical or immunological response.

#### Discussion

DR. C. HENRY KEMPE, Denver: Since Dr. Katz' paper was prepared, we have vaccinated an additional 20 children with the Edmonston strain and have obtained identical results. Among 40 children, only one has had a subclinical immunization while all others have had either fever or a mild rash or both. None have had any catarrhal symptoms or encephalitis. We have asked over 100 pediatricians if they would be prepared to use a measles vaccine which caused predictable mild illness and all but three thought they would be pleased to have such a product. Mothers have never been known to get excited about measles; most are quite unaware just how serious and disabling measles can be. The use of measles vaccine, therefore, may well need the persuasion of the pediatrician.

We have challenged five of the immunized children with attenuated virus and they failed to get the disease. On the other hand we have similarly challenged a group of 15 children, immunized with live distemper virus some time before, and 3 of these have promptly got modified measles and were thus not protected by their distemper virus injection.

DR. J. CHR. SIEM, Copenhagen, Denmark: May I thank you very much for your valuable contribution. When measles occurs in isolated communities, the disease may constitute a serious problem, such as was shown by Panum in his classical epidemiological study of measles in the Faroe Islands in 1846. This was also the case in the Greenland epidemic of 1951, where a total of 4,320 persons were attacked, and the morbidity rate was 99.9%, the mortality rate 1.8%, and complications occurred in 45% of the cases (Christensen et al.)

I was intrigued by the fact that Koplik spots were seen in only one of the vaccinated, and I wonder whether you have observed leukopenia, and whether the thymol turbidity test has been positive as it is in the natural disease.

* By invitation.

Finally, I would like to ask you whether you have tried vaccinating by the natural route, e. g., by spraying with the virus suspension.

DR. ALFRED L. FLORMAN, Manhasset, N. Y.: I wonder in view of the infrequency of Koplik spots and rash in your vaccinated children, whether you made nasal smears and looked for the large multinucleated "measles" cells. It has been reported that these cells can be demonstrated in patients given sufficient γ-globulin to prevent both Koplik spots and rash.

DR. PAUL WEHRLE, Syracuse, N. Y.: Since a rash was present in some of the patients and Koplik's spots were seen in at least one, I wonder whether the failure to demonstrate virus appearance in the blood might perhaps be due to the adaptation of the virus to chick cell or the timing of collections rather than an absence of viremia.

DR. DAVID KARZON, Buffalo: One of the important complications of measles we all fear is encephalitis. Do you have any other evidence, in addition to the intracerebral inoculation of the several monkeys which you cited, which would lead you to believe that the incidence of encephalitis would be lower than the expected approximately one in a thousand? It is known that even a mild measles infection may be encephalitigenic.

DR. JOSEPH STOKES, Philadelphia: In exposed children who have had γ-globulin in small amounts for attenuation and in whom there has been mild fever and moderate rash, have you had any indication whether antibodies appear and whether they remain elevated? Also have you followed such cases in order to determine whether their immunity remained over many years? You may recollect that in studies with measles virus grown on the chorioallantois of the check embryo rapid attenuation of virus was obtained. In those cases receiving the early passage material and in whom mild disease with rash occurred there was resistance after exposure to measles in the early years after vaccination. Later, however, these same children did have mild measles or clinical measles after exposure. Thus immunity did not last even in those children who had attenuated disease from early passage virus.

DR. KATZ: Thank you all very much for your expressions of interest in our presentation. First, Dr. Kempe, I am glad to know that 20 more children have been vaccinated with the same results. I think the proposed experiment with canine distemper vaccine is an important one. We do not have sufficient time today to discuss the question of the relationship of distemper and measles viruses. I can only say that from our own experience and that of Dr. Cabasso at Lederle Laboratories it is difficult to imagine that immunization with distemper virus will produce antibodies in human beings protective against measles. There are certainly some laboratory similarities between measles, distemper, and rinderpest viruses, but whether crossing occurs of sufficient quantity or quality to offer reciprocal immunity seems very doubtful. It is hard to conceive why in countries like our own where distemper virus is prevalent that measles would still exist as a major childhood disease if prior exposure to distemper would protect against it.

Dr. Siim, I was pleased to hear your remarks and I think they point up one of the aspects of measles which we overlook in this country. Even though approximately 500 children die annually in the U. S. A. from measles and its complications, this does not represent anywhere near the morbidity and mortality in such areas as South Africa, Greenland, Yugoslavia, and other nations, where one finds large isolated populations suddenly exposed to the disease or childhood groups with high mortality from secondary infection. Physicians who work for the W. H. O. have repeatedly pointed out to us what a major role measles plays in deaths from exacerbated tuberculosis in the areas where tuberculosis remains a large childhood problem.

In regard to leukopenia, we have tested the monkeys, and, although in any individual case it was difficult to be certain, a geometric mean of the entire group showed definite leukopenia at the expected time. With our initial group of vaccinated children, we did white blood counts, and here again the trend was not evident with individuals but seemed definite with an over-all group calculation.

We do not recommend using the nasal route of vaccination for two reasons. First, we were unable to infect susceptible monkeys with attenuated virus via nasal instillation. Although the agent persisted in their nasopharynx for three to five days, it never seemed to invade and did not produce an antibody response. Secondly, if there is any danger of encephalitis from direct viral invasion of the brain, the proximity of the cribriform plate to the nasopharynx makes us wary. In this country physicians are accustomed to giving subcutaneous immunization, and this is perhaps a third reason why we felt that route would be most readily accepted and effectively performed. Spraying a lot of virus around one's office might expose children whom one did not intend to vaccinate on that occasion.

Dr. Florman, we did not do nasal smears on either the children or the monkeys. Neither group, however, showed signs or symptoms of respiratory tract involvement, that is, no cough, coryza, or conjunctivitis. Attempts to recover the virus itself from the nasopharynx have been uniformly unsuccessful in the vaccinated subjects.

Dr. Wehrle, your point regarding the systems in which we test for virus is an important one. We have been able to propagate chick virus easily in both human amnion and human kidney cell cultures. It produces the expected cytopathogenic effect in either. We also mixed chick virus with normal monkey blood and human blood, and in both instances it was still productive of the measles cytopathology in amnion and kidney cultures. In earlier experiments we used chick cell cultures as well in parallel isolation attempts and found kidney cultures more sensitive.

Dr. Karzon, we do not know what causes measles encephalitis, but are inclined to lean toward the view that in most cases it is an allergic type response. For lack of any other test, we have done direct intracerebral inoculation of five monkeys with the measles virus. In no case was a neuropathologist able to detect any microscopic evidence of CNS involvement. To my knowledge there is only one reported case of virus recovery from the brain of a patient with measles encephalitis. This was described by Shaffer, Rake, and Hodes. At the time this work was done, monkey inoculation was the only way of testing for virus. Since that time, the varying reliability of this method has been demonstrated, and we must have a great deal of admiration for those who worked under these adverse circumstances. However, many of the results obtained by this tedious method now seem open to question.

Dr. Stokes, our experience with γ-globulin modification of measles is no different than anyone else's. We believe that normal human beings obtain life-long immunity after one attack of naturally acquired measles. On the other hand, the incidence of second attacks in children whose original disease was modified by γ-globulin ranges from 1 in 100 to 1 in 10, depending on whose statistics one studies. We have not had an opportunity to study thoroughly the antibody response in γ-globulin modified measles though we intend to do so.

We are familiar with the results obtained when your vaccinated subjects were naturally or deliberately challenged with measles. Unfortunately, however, there were no laboratory tests available at that time to determine the presence or absence of specific antibodies in the children whom you had vaccinated. We are encouraged by the antibody titers maintained by our initially vaccinated children over a six-month period. Studies by Dr. Black in New Haven, Dr. Ruckle, when she was in Pittsburgh, and in our own laboratory have all shown that the antibody titers three months after natural measles remain at nearly the same level for life. Therefore, we would hope that the titers six months after vaccination in our study group might also remain unchanged.

## 74. The Clinical and Laboratory Findings of Premature Infants Fed a Concentrated Form of a Low-Solute-Content Breast-Milk Substitute

H. G. Keitel,* R. Ting,* L. Schlitt,* H. Moon,* A. Price,* R. Cohn,* T. Liddy,* D. Harnish,* and R. Bernardin,* Jefferson Medical College
Introduced by Lewis Barness

It has been appreciated for many years that infants are susceptible to dehydration because of their relatively high rate of water loss. An attempt was made to increase the *water reserve* of premature infants by diluting the powdered form of a standard breast-milk-substitute formula (Similac). It was assumed that the infants, who were allowed to drink as much formula as they desired, would increase their intake (cubic centimeters per kilogram per day) to sustain their *caloric* intake at a "normal" level. However, most of the premature infants drank the same volume of dilute formula as the regular formula. The dilute formula contained from 10 to 15 Cal. per ounce, and the regular dilution, 20 Cal. per ounce. Consequently, the caloric intake of the infants fed dilute formula fell.

Recognizing that the caloric intake of premature infants frequently is very low—particularly in the first three weeks of life—and that according to the data of Scammon and of Yippö the rate of weight gain of premature infants is less than the in utero rate, it was decided to use a concentrated form of the low-solute-content milk. This formula contained 40 Cal. per ounce. Most of the infants drank about the same volume of the concentrated formula (cubic centimeters per kilogram per day) as of the regular formula; consequently, the caloric intake greatly increased. Evidence of intolerance was encountered infrequently.

In infants receiving the regular or the concentrated form of the milk, the intake rose gradually after birth from 150 to 350 cc. per kilogram per day by the second week. Thereafter the daily intake declined only in infants whose body weight rose over the projected in utero level. The rate of weight gain of infants receiving the regular dilution of the formula rarely resulted in their catching up to the projected in utero weight level, whereas the body weight of the infants who received the concentrated formula often reached or exceeded the projected in utero level before discharge (2,500 gm.).

The findings of over 150 premature infants who were fed a concentrated formula will be presented and compared to the findings of infants fed the regular dilution of the formula. The findings include (1) the rate of weight gain and the rate of growth, as determined by multiple consecu-

* By invitation.

tive body length and head- and chest-circumference measurements; (2) length of hospitalization required to attain a weight of 2,500 gm.; (3) plasma and urinary osmolar concentrations; (4) the albumin, globulin, chloride, $CO_2$ and $\alpha$-amino acid nitrogen concentration of plasma; (5) morbidity and mortality results; (6) photographs at birth and on discharge.

The most striking finding in the infants fed the concentrated formula was a rapid rate of weight gain associated with an *appropriate* increase in growth. The weight gain was directly correlated with the caloric intake.

Some of the infants weighing less than 1,500 gm. at birth became chubby, but others remained lean, even though their caloric intake had ranged from 200 to 350 Cal. per kilogram per day and their rate of weight gain was double the rate that is usually seen when "isocaloric" formulas are offered. The finding of lean and chubby infants was considered to represent a normal range of findings.

It will be shown that caloric deficiency constitutes a great threat to the water status of the premature infant and that the use of a concentrated formula serves to reduce this hazard.

A group of infants who received the regular dilution of the formula supplemented with sodium chloride to bring the sodium content to the level of the concentrated formula temporarily had a large rate of weight gain, but by the time of discharge (2,500 gm.), the rate of weight gain for their entire nursery course was the same as the infant who received regular formula without added sodium. This finding and the failure to observe edema except in the first week or two of life (when infants who receive regular formula also frequently have mild edema) indicate that an inappropriately large accumulation of extracellular fluid does not account for the large rate of gain of the infants offered concentrated formula.

The data appear to be best interpreted as follows: When regular, "isocaloric" formulas (20 Cal. per ounce) are offered, the premature infant frequently is limited from obtaining enough food (calories) because of gastric limitation for volume. Consequently, he suffers from malnutrition and growth retardation. The use of a concentrated formula tends to overcome this physiologic handicap.

### 75. Antibody-Resistant Vaccinia Necrosum Cured by Passive Transfer Sensitization

C. HENRY KEMPE and ALTON J. MORRIS,*
University of Colorado

A case is presented of a child with progressive vaccinia necrosum whose course strongly suggests that virus antigen sensitization may be an essential defense mechanism in localizing virus infections.

A one-year-old child experienced a progressive vaccinia lesion extending over his shoulder and chest wall three months after his primary vaccination. Despite a normal γ-globulin level, he was found to have no neutralizing or hemagglutinating antibodies. He also showed no skin reaction to killed vaccinia virus. Investigation showed that his white blood cells were able to phagocytize vaccinia virus in a normal fashion. He showed no clinical response to administration of massive doses of antibody, resulting in titers well in excess of those obtained by normal children after primary vaccination. With the absence of response to passively administered antibodies, simple debridement and finally radical forequarter amputation were done without arresting progression of the process. As the lesions recurred at the edges of the resulting massive skin defect, passive transfer sensitization was attempted by the injection, around the entire involved skin area, of sensitized white blood cells from recently vaccinated adult donors. At the same time three lymph nodes from a recently vaccinated donor were transplanted into the rectus muscle and a cell suspension of three additional lymph nodes given intravenously. Local sensitization to killed vaccinia virus was demonstrated, and the lesions regressed in a dramatic fashion and have not recurred in the subsequent year.

This case permits clear differentiation between the effects of antibody and of sensitization and strongly suggests that virus sensitization may be an essential defense mechanism, possibly more important than humoral antibodies or white blood phagocytosis.

### 76. Disturbances of Sodium and Water Homeostasis in Hypernatremic Infants

GERALD A. KERRIGAN, Marquette

Eleven infants, 3,000 to 11,000 gm. in weight, with hypernatremia due to gastroenteritis, have been studied by balance techniques for two to six days. Initial serum sodium concentrations were between 150 and 190 mEq. per liter. Intravenous fluids having 150 mOsm. per liter were given at rates providing approximately 4 to 8 mEq. Na per kilogram per day. After the initial voiding potassium was provided at slightly lower rates. When the babies were able to take fluids orally, half-strength boiled skimmed milk was offered. Sodium bromide was used to measure extracellular volume on one or more occasions in six infants. Changes in extracellular volume have been calculated from the measured volumes by means of chloride space. Total body water has been assumed to be 70% of final body weight.

* By invitation.

During the first 24 hours all 11 babies had positive sodium balances. The least severely ill infant reached sodium equilibrium at the end of 24 hours. The other 10 babies retained sodium for periods up to 96 hours. Sodium retention ranged from 8 to 14 mEq. per kilogram.

There are reasons to consider sodium retention by at least five of the babies to be inappropriate. Two infants became edematous; two were observed to have subsequent negative sodium balances not due to low oral sodium intakes, and four retained sodium despite high initial values of 50 to 63 mEq. per kilogram for total bromide-space sodium. In one of the five, positive sodium balance was associated with renal sodium conservation. In the others positive sodium balances were associated with slowly rising rates of renal sodium output and would seem to be due to slow adaptation to rates of sodium intake.

Six convulsive episodes occurred in 5 of the total 11 infants. In three instances seizures were associated with sodium retention and expansion of measured bromide spaces by transfer of water from the cellular compartment. Minimum intracellular volumes of 25% to 29% of body weight were then observed. In these instances urine solute concentrations were equal to or greater than the solute concentrations of the initial voidings, suggesting lack of available surplus water. Six babies who did not convulse formed urine more dilute than the initial specimens soon after therapy began.

Two of the babies who convulsed in association with transfer of water from the cellular to the extracellular compartment died. One brain was not examined. The other did not show gross hemorrhage. Microscopically the blood vessels were congested and there was extravasation of red cells into the meninges.

Three of the six convulsive episodes were due to water intoxication. In these instances intracellular volumes 110% to 140% of the final values were observed. In each instance the urine was maximally concentrated. Coincident serum solute concentrations in two infants were below 260 mOsm. per liter. In the third, overexpansion of intracellular volume occurred when the serum solute concentration was 360 mOsm. per liter.

These clinical observations indicate that convulsions may occur in hypernatremic infants when abnormally small intracellular volumes are caused by transfer of water to extracellular fluid, as Finnberg has pointed out. Water intoxication, however, is also an important cause of seizures in hypernatremic babies. Further, movement of water out of cells is sometimes due to inappropriate retention of sodium when surplus water is not also available. On the other hand, movement of water into cells is sometimes due to inappropriate retention of water. Inappropriate retention of sodium and water suggests that the functional lesion responsible for seemingly paradoxical sodium and water homeostasis in some hypernatremic babies may be a small effective vascular volume.

### 77. The Persistence of Virus in the Environment of Patients with Kaposi's Varicelliform Eruption

SIDNEY KIBRICK, Harvard

Kaposi's varicelliform eruption (eczema herpeticum or vaccinatum) has been reported in eczematous children following their hospitalization in rooms recently or concurrently occupied by patients with this syndrome. The present study was concerned with attempts to determine, by tissue-culture techniques, the persistence of infective virus in such rooms.

Sweepings were obtained at varying intervals from the rooms of seven patients with this disorder (five with eczema vaccinatum and two with eczema herpeticum). In each instance the responsible agent could be isolated with ease throughout the period of hospitalization. Despite various procedures of terminal disinfection, viruses were also recovered from rooms previously occupied by three patients with eczema vaccinatum and two with eczema herpeticum for as long as 14 days after discharge.

In view of the persistence of vaccinia and herpes simplex viruses in these environments, the inactivating effect was determined of certain physical and chemical agents on the infectivity of dried exudates, flakes, and crusts derived from patients.

Vaccinial crusts stored at room temperature were found to contain infective virus, occasionally in relatively high concentration, for periods up to three years—the longest interval studied. When stored in finely pulverized form, however, the infectivity of such materials was less persistent. The virus, both in whole and in freshly pulverized crusts, was resistant to high concentrations of a detergent-iodine complex commonly employed for hospital disinfection, despite prolonged immersion, and withstood high intensity ultraviolet irradiation for up to five days.

Herpes simplex crusts, when stored intact but not when finely dispersed, were infective in some instances for at least several weeks. The results of additional studies with this agent, currently in progress, will also be reported.

Varicella in the patient with eczema may occasionally mimic Kaposi's varicelliform eruption. Attempts to recover this virus from freshly desquamated scabs were uniformly unsuccessful, although it was easily demonstrated in cultures inoculated with vesicle fluid.

The results of this study permit the more exact formulation of recommendations and procedures

for reducing the incidence of Kaposi's varicelliform eruption in susceptible patients during hospitalization.

### Discussion

DR. C. HENRY KEMPE, Denver: We believe that about 10 to 12 such cases of hospital-acquired eczema vaccinatum occur each year. Children with eczema should generally not be admitted to the hospital for the dual reasons of being at risk from vaccinia virus and also because they are massive skin dispensers of staphylococci. The data presented by Dr. Kibrick nicely dovetail with findings on variola. Scabs from smallpox patients retain their virus titer at room temperature for several years provided they are kept reasonably dry. I wonder if the protein protection of dried plasma in smallpox and vaccinia scabs is not responsible for virus survival?

DR. HORACE L. HODES, New York: I should like to ask a couple of questions. One is whether you have had any experience with wet vacuum cleaning? This might be a more effective method. The next question is a technical one, having to do with the method used to show whether or not the virus was present. Did you grind up the crusts or pulverize them and then test them, or were the untreated crusts put directly into tissue culture? The implication here is that the crusts might seem to be more infectious than they actually are if pulverization were used.

DR. ROBERT B. LAWSON, Miami, Fla.: Dr. Kibrick nicely showed how dangerous the room is. I'm wondering whether they have been able to make any experiments to show how far away from the room the virus also may be dangerous by virtue of footwear transport.

DR. JOSEPH STOKES, Philadelphia: I would be interested in knowing more about the size of the dust particles, as to how many micra in diameter they were, and how you pulverized the particles in order to determine that ultraviolet light did not affect them.

DR. SAMUEL KARELITZ, New York: Since some recently vaccinated children must be admitted to a hospital for one or another reason and since Dr. Kibrik has demonstrated the persistence of the vaccinia virus in hospital rooms presumably well cleansed, might it not be wise to vaccinate infants very early in life, possibly even the first week of life. This would reduce the danger from acquiring generalized vaccinia in a hospital.

DR. MAXWELL STILLERMAN, New York: If the crusts of children vaccinated against smallpox contain vaccinia virus, how do you explain the recommendation of the American Academy of Pediatrics that vaccinated children be separated from siblings with eczema for only 10 days?

DR. HEINZ F. EICHENWALD, New York: I'd like to ask Dr. Kibrick if he has been able to show that this material can be carried on the clothing of the nurses and doctors from one patient to another.

DR. KIBRICK: Dr. Kempe, we have no experimental data on the mechanism of virus preservation in these crusts. Storage of crust material in the pulverized state results in a more rapid loss of infectivity, however, than when the crusts are stored intact. This would suggest that the crust constituents, which are primarily dried plasma proteins, form a protective coating around the virus particles, and the thicker or denser this coating, the longer the material remains infective.

Dr. Hodes, we have had no experience with either vacuum cleaning or water vacuuming of infected rooms. Either would appear to be preferable to mopping. Liberal applications of a germicidal detergent followed by water vacuuming or preceded by dry vacuuming would both be more satisfactory, in my opinion, than any mopping method of cleaning the rooms.

Our techniques for isolation of virus from these rooms have undergone several modifications during this study. Initially, sweepings were ground with culture media and the centrifuged supernates were used as inocula. Subsequently, the sediments were also tested as routine, since these were often positive when the supernates were negative. More recently, the sweepings have been examined with a hand lens, and suspicious particles, only, removed for testing, a procedure which reduces the incidence of toxicity. Since vaccinia can withstand short exposures to trypsin without appreciable loss of titer, sweepings from vaccinial rooms have also been tested by grinding with 0.25% neutralized trypsin followed by incubation of the mixture for 30 minutes at 37 C. This results in release of virus from any crusts or flakes which may be present. The action of trypsin is then stopped with soy bean inhibitor, the mixture centrifuged, and both supernate and sediment are used as inocula.

While it might be supposed that crust-encased virus carries little risk to a susceptible patient, we have found that infective virus can be liberated from such crusts or flakes by exposing them to fresh serum, presumably as a result of proteolytic action. It is possible, therefore, that virus may be liberated in this way from flakes or small particles which land on patients with open or eczematous skin areas. We have no data on the risk from inhalation.

Dr. Lawson, we have attempted on several occasions to demonstrate virus in sweepings of adjacent corridors and rooms but have not been successful except in those instances where one of these patients has previously occupied the room.

The comments of Dr. Robbins, above, are of interest in this regard.

Dr. Stokes, we did not measure the size of the ground crust particles that were exposed to ultraviolet irradiation. Our experiments with ultraviolet were quite crude and consisted of exposing both ground and intact crusts to prolonged irradiation and then testing for infective virus. In most instances the preparation remained infective.

Dr. Karelitz, I would agree with your comments on the advantages of early vaccination. As far as herpes simplex is concerned, this agent is of course widely distributed, and the risk of herpetic infection is probably as great for nonhospitalized susceptibles with eczema or other skin lesions as for those that are hospitalized. In view of the severity and mortality of eczema herpeticum, however, contact of these patients with known herpetic infections should still be avoided wherever possible.

Dr. Stillerman, Dr. Charles Pryles and I recently demonstrated vaccinia virus in the pharynx of a child 11 days after an uncomplicated primary vaccination. In view of this, I would not feel that the recommendation that children undergoing vaccination be separated from siblings with eczema for a period of 10 days affords an adequate period of safety.

Dr. Eichenwald, we have no data on the transport of these agents on the clothing of hospital personnel.

### 78. Enzymatic Detection of the Galactosemic Trait

H. N. KIRKMAN,* National Institute of Arthritis and Metabolic Diseases
Introduced by Victor A. Najjar

Galactosemia is a disease manifested by a marked deficiency or absence of activity of the enzyme galactose-1-phosphate uridyl transferase, which catalyzes the reaction: (*a*) galactose-1-phosphate +uridine diphosphate glucose⇌glucose-1-phosphate +uridine diphosphate galactose. The disease is generally regarded to have a simple recessive mode of inheritance in which the parents, although apparently healthy, should carry one normal and one abnormal gene for the condition. A corresponding partial impairment of galactose metabolism in some, but not all, such heterozygous persons was found by Holzel and Komrower through the use of galactose-tolerance tests. In order to compare as accurately as possible the galactose-1-phosphate uridyl transferase activities of normal and heterozygous blood, a new method (*Ann. Human Genet.* 23:117, 1959) has been devised which permits continuous following of Reaction (*a*) under conditions yielding zero-order kinetics, initial rates, and an accuracy of 5% to 8%. The assay has as its basis the manometricly observable oxygen consumption occurring in hemolysates subsequent to the generation of glucose-1-phosphate by Reaction (*a*). Earlier evidence (Conference of Enzymes in Blood, Feb. 1958, *Ann. New York Acad. Sc.* 75:274, 1958) at an enzymatic level of a galactosemic trait in persons presumably heterozygous for galactosemia was made possible through this method. Subsequent refinements and extension to a study of 18 parents revealed that all of the parents had activities below the mean for 18 normal controls. Thirteen of the eighteen had activities more than 2 standard deviations below the mean for normals. Difference of the means is significant at the $P<<0.001$ level. The mean for parents (±S. E. of mean) represents 63% (±4%) of that for normal controls. This method has made possible the tracing of affected or heterozygous persons through four generations of one family and provides an impetus for more extensive family studies by this or alternate means of estimating the enzyme of (*a*). Reiteration is made of the earlier warning by the originators (*J. Lab. & Clin. Med.* 50:469, 1957) of the uridine diphosphate glucose consumption test against use of the unmodified test for demonstrating the galactosemic trait. Deliberately devised overincubating and overloading with hemolysate brings Reaction (*a*) almost to completion when normal bloods are used in that test. This makes definitive the contrast between normal and galactosemic bloods but serves, along with intrinsic sources of error, to obliterate moderately reduced activities.

* By invitation.

### 79. Diverse Effects of Cortisone upon Phosphate Metabolism

R. KLEIN, Pittsburgh

Balance studies were done on a patient with hypoparathyroidism and on one normal control. The effect of 150 mg. of cortisone by mouth per day for three days, before and after the administration of vitamin D, was studied in the patient with hypoparathyroidism. The normal 14-year-old boy was given 200 mg. of cortisone by mouth per day for three days. Urine, stool, and serum phosphorus were measured. Cortisone administration to the patient with hypoparathyroidism was accompanied by increased intestinal absorption of phosphorus, decreased urinary excretion, and a rise of serum phosphorus from 11.3 to 15 mg. % before he was given vitamin D. There was no rise in serum phosphorus when the tests were repeated while the patient was receiving vitamin D in doses of 100,000 units per day. Cortisone administration in the normal boy was unaccompanied by any change in urinary or stool phosphorus. There was a slight rise in serum phosphorus.

Twelve white male rats were given a synthetic diet from which they obtained less than 15 mg. of

phosphorus per day. After five days on the diet, they were placed in individual metabolism cages and urine and stools were collected. Blood was obtained at the end of the experiment from the abdominal aorta. Six of the animals were given 10 mg. of cortisone intraperitoneally each day for the three days of the experiment. This dose produced marked phosphoturia in similar rats given a normal diet and in parathyroidectomized rats. The control animals were given injections of an equivalent amount of saline. All the rats excreted only minute amounts of phosphorus in their stools or their urine. There is no difference between the treated and the control groups in phosphorus excreted or in serum phosphorus levels.

These experiments were carried out with two purposes in mind. One was to establish whether the clinically deleterious effects of cortisone on phosphorus metabolism that were reported to these meetings several years ago in a patient with Addison's disease as well as hypoparathyroidism existed in the patient with hypoparathyroidism and no Addisonian symptoms. This was established. The other was to explain the discrepancies between these findings and the phosphaturic effect of cortisone described in humans by Ingbar and in dogs by Pitts. This was confirmed in rats by Laron, at the same time that we also demonstrated increased intestinal absorption of phosphorus in rats given cortisone. The same phosphaturia was found in parathyroidectomized rats who were fed normal diets and given cortisone, but there was no phosphaturic effect of cortisone in starved parathyroidectomized rats. From the evidence at hand we can only suggest that the difference in the results is a question of dosage. Ingbar needed three to five times as much cortisone as we gave our patients to produce phosphate diuresis in normal humans. The doses used by Pitts and in our own investigations in animals were of the same or of a greater order of magnitude. All the doses used, however, demonstrated a similar increase in phosphorus absorption in the intestine. Still unanswered is how cortisone causes increased phosphate reabsorption by the tubules in small doses and in large doses leads to decreased phosphate reabsorption by the renal tubules. Presumably there is a secondary mechanism in this, since this latter effect does not occur unless phosphate absorption occurs from the intestine. This mechanism is not known at present.

### 80. Mechanisms of Therapeusis in Hyperkalemic Familial Periodic Paralysis

R. KLEIN and T. EGAN,* Pittsburgh

Treatment of paralysis in this disease is accomplished by a number of agents, among which glucagon and epinephrine are the prototypes. Glucose has some prophylactic effect, and we have prevented attacks in susceptible persons by the daily administration of *d*-amphetamine during the past year. The substitution of a placebo or the suspension of administration of *d*-amphetamine has led to the recurrence of paralytic attacks.

Last year at Atlantic City we reported changes in potassium concentration with attacks, and this year we have described shifts in water from the extracellular to the intracellular compartments and a depression of muscle potassium.

We have demonstrated effects of therapeutic agents solely upon potassium shifts. Epinephrine, *dl*-amphetamine, and glucagon, when given during paralysis, raise blood sugar and increase peripheral uptake of glucose as they do when given to normal persons. At the same time they produce a greater fall in arterial plasma potassium in patients than in normals. Epinephrine and *dl*-amphetamine produced a depression of peripheral uptake of potassium and at times were actually accompanied by a negative peripheral A-V difference, indicating peripheral release of potassium. On one occasion, glucagon was followed by a slight increase in peripheral uptake of potassium.

Glucose and glucagon administered after spontaneous recovery from paralysis were followed by a loss of potassium from peripheral tissue. When glucose was given in the interval between attacks, there was a greater fall in arterial potassium than in venous potassium. It was impossible to demonstrate an effect of glucose ingested during an attack.

Intravenous administration of hydrocortisone hemisuccinate during the asymptomatic state lowered plasma protein and increased plasma water slightly.

Prophylactic administration of *d*-amphetamine resulted in reversion of the EEG to normal. It did not prevent secretion of salt-losing hormone.

The weak prophylactic effect of glucose presumably is brought about by its action in minimizing glycogenolysis and the potassium elevation that normally accompanies this. The beneficial effect may be tempered by release of this ion from the periphery.

The beneficial effect of epinephrine and its analogues results from the lowering of arterial potassium, although there is no apparent uptake of potassium by cells and so the lowered concentration of muscle potassium is not improved.

#### Discussion

DR. MALCOLM A. HOLLIDAY, Pittsburgh: Dr. Klein, can you tell us any more about the possible effects of the salt-losing material in producing the paralysis?

DR. INGRID GAMSTORP, Lund, Sweden: This disease is probably far commoner than we formerly believed. The symptoms are so slight and the

* By invitation.

patients so accustomed to being considered hysterical that they tire of seeking medical help. I was very much interested in hearing of the combination of paralytic attacks and something suggesting myotonia in your patient. Last summer Dr. Milton Shy described a family with paramyotonia, paralytic attacks, and changes of serum potassium. On the other hand, I have not found signs or symptoms of myotonia in any of my 68 patients with paralytic attacks. I have another family with classical paramyotonia, and these patients' reaction to potassium is quite different from that of patients with adynamia episodica hereditaria. However, the description of your patient's symptoms suggests that he had myotonia also, and I wonder if you have been able to demonstrate this clinically or electromyographically.

I was also very interested to hear of your successful attempts at treatment. When I left my country, I promised my patients that I would try to bring home some new methods for treatment. It appears as though I will succeed.

The serum sodium was determined twice an hour during the course of some 60 attacks in 17 patients without any changes being found, so I feel certain that the serum sodium neither increases nor decreases during attacks.

As to the name of this disease, I dislike the term hyperkalemic familial periodic paralysis as it fails to take cognizance of the fact that the patients may have attacks without hyperpotassemia. It is true that the serum potassium almost always increases during the attacks but in almost a third of the 60 or so attacks that I have studied in 17 patients it never exceeded the upper limits of normal and thus there was no hyperpotassemia. Furthermore, although the increasing serum potassium accompanies the weakness, I do not believe it is the cause of the weakness. Until we become sure of this point, I think it is wiser to omit the word potassium in the name, particularly as the disease already has the descriptive name of adynamia episodica hereditaria.

I was usually able to provoke attacks in fasting patients by giving them 3-5 gm. of potassium chloride by mouth, but if they were given a 100 gm. of glucose about half an hour before the potassium, the attack was prevented. The patients tell us that they feel better if they are allowed to eat many small meals, and that they are more liable to have attacks if they are hungry. These observations are in accord with yours.

DR. LAURENCE FINBERG, Baltimore: Could you give us some speculation as to why amphetamine might work on these patients?

DR. LYTT I. GARDNER, Syracuse, N. Y.: I wonder if Dr. Klein would be willing to speculate on the possible chemical structure of his sodium-losing material?

DR. KLEIN: First let me reply to Dr. Gamstrop, ladies first, and we owe her a great debt. No, we have not done any electromyography or any examination of this sort.

I don't mean to start a polemic on the name. We chose this because these patients have been reported for many years as instances of atypical familial periodic paralysis that did not respond to potassium administration. Since this confusion already existed in the literature, we attempted merely to segregate these patients from the whole group of patients with similar clinical symptoms. We have an idea that, as Dr. Gamstrop mentioned, this type probably constituted a large percentage of the patients with familial periodic paralysis. I am willing to compromise until we know the exact etiology and call this Gamstrop's syndrome.

Our patients, too, found eating frequent high-carbohydrate meals was helpful. I think that glucose does not work therapeutically because, though it does cause a drop in plasma potassium presumably through visceral uptake, it does not seem to have any effect on the peripheral uptake. This is purely speculative.

In answer to Dr. Finberg, we think that we have a rational basis for our therapy. We have assumed that the ratio of extracellular and intracellular potassium, if not the all-important factor, was one of the most important factors in this disease. Therefore, we have used agents that lower the serum potassium and are known to increase peripheral uptake. That's why we started off with epinephrine, which was unwieldly. We can see no difference in the action of the two except that *dl*-amphetamine has very little effect on glucose changes. It has some, but as you recall the literature, it has very little.

Dr. Holliday, in one sense our basic hypothesis is that the concentration of intracellular potassium is of utmost importance here. Our meager evidence on biopsy showed that the intracellular potassium could be low between attacks. We feel that this is the effect of constant or intermittent secretion of salt-losing hormone, which acts antagonistically or in the opposite direction to aldosterone. Aldosterone will increase muscle potassium and lower muscle sodium, and we believe this material does just the opposite. Then when serum potassium increases for any of the other reasons we mentioned or intracellular potassium concentration is further lowered by water and sodium entering the cells, paralysis ensues. Thus, the salt-losing material acts as a permissive factor.

Dr. Gardner, as you know, we measure this material by bioassay. Neher and Weltstein have recently isolated 16 hydroxypregnanes from various salt-losing syndromes. This may be the material we have, since their paper chromatographic results were not unlike ours and our infrared patterns

were seen by them and they considered them comparable to their own infrared results. Now, the materials that have been tried so far, the synthetic materials, have not been salt-losing. However, there are many possibilities here. I would think that at the present time at least we would feel that this material probably is in the 16-hydroxypregnane group or runs on paper with it. This speculation is not very secure. They have found it as we have in newborn infants, adrenal hyperplasia, and we have also found it in the urine of a normal adult whose salt intake is something just short of miraculous.

### 81. Properties of Infectious Ribonucleic Acid from Highly Purified Poliovirus Tested in Human Cell Monolayers

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OLGA VAN DAMME,* and HATTIE E. ALEXANDER,
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Certain properties of infectious ribonucleic acid (RNA) obtained from highly purified poliovirus can be studied quantitatively on cell monolayers prepared from a number of human cell lines. The number of plaques induced by free RNA is strongly dependent on (*a*) pretreatment of the cell monolayers with hypertonic solutions, (*b*) the ionic strength, and (*c*) the pH of the solution in which the RNA is applied. Provided these three factors are properly controlled, the number of plaques formed are proportional to the RNA dilution factor, making quantitative studies on the biological activity and physicochemical stability of RNA preparations feasible. On the other hand, incubation of cell monolayers at different temperatures before and after seeding with RNA and seeding at temperatures between +4 C and +36 C showed no influence on the rate of uptake of the RNA by the cells and yielded the same number of plaques after further incubation at 36 C. The number of plaques induced by a given amount of RNA was not influenced by washing the cell monolayers with different salt solutions after infection with RNA and subsequent 30-minute incubation at room temperature.

The infectivity of the isolated RNA is, even under proper conditions, only 1/100th that of the virus solution from which the RNA was prepared, but the degree of infectivity of free RNA prepared from crude infective tissue culture fluid, partially purified poliovirus and highly purified poliovirus, is the same in our test system. This finding contradicts the hypothesis that cellular components present in virus preparations are important for infection by RNA.

Furthermore, it was observed that RNA obtained from poliovirus preparations which differed considerably in their degree of purification was equally sensitive to inactivation by ribonuclease and equally stable to storage at low temperatures and exposure to high temperatures, indicating that cellular components present in crude virus preparations do not protect the RNA against damage during the preparation procedure or during the period of invasion and infection of the cells.

The infectivity of the isolated RNA shows no decrease upon storage at +4 C over a period of a month or upon exposure to +48 C for 2 hours or +100 C for one minute. RNA preparations heated to +48 C for two hours are, however, unstable when stored at +4 C.

* By invitation.

### 82. Bilirubin Inhibition of Heme Biosynthesis

ROBERT F. LABBE,* MERLIN R. ZASKE,* and
ROBERT A. ALDRICH, University of Washington

Recent experiments in this laboratory have shown that the last step in heme biosynthesis, i. e., the insertion of iron into protoporphyrin, is catalyzed by a soluble enzyme obtained from several different tissues in the rat and the chick embryo (*Biochim. et biophys. acta,* to be published). Since the substrate, protoporphyrin, has identical propionate side-chains with bilirubin which can exist in similar spatial arrangements, one can readily imagine how bilirubin might compete with protoporphyrin for the enzyme. Reduction of the γ-methene bridge between the pyrroles bearing the propionate groups would have little effect on their relative spatial positions. Any effect of this competition would probably be inhibition of heme synthesis.

The soluble enzyme preparation used in these studies was obtained from rat-liver mitochondria and its activity assayed as reported in the above publications except that the reaction was stopped after 20-minute incubation. Bilirubin (Eastman Kodak Company) was dissolved in 10% sodium carbonate, adjusted to pH 8 with HCl, and diluted to a concentration of 0.007 M. In this system bilirubin inhibited the utilization of protoporphyrin for heme synthesis and paralleling this there was a decrease in the uptake of $Fe^{59}$. Preliminary kinetic studies showed that by varying the concentration of protoporphyrin and bilirubin while the iron remained constant there appeared to be competitive inhibition. Bilirubin is an active inhibitor of this reaction, since 50% inhibition was obtained at a bilirubin concentration of $10^{-5}$ M and with a bilirubin-protoporphyrin molar ratio of 53.

The same reaction has been studied with use of rat-brain homogenate and bilirubin inhibits in this preparation also. This data does not explain the clinical manifestations of hyperbilirubinemia but suggests that one action of bilirubin may be inhibition of the biosynthesis of heme, the prosthetic group of several enzymes essential in biological oxidation.

### 83. The Intravenous Injection of Fluorescein-Labeled Antibody as Means to Localize and Quantitate Antigen Location

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Introduced by A. Leonard Luhby

The γ-globulin fraction of anti-rabbit-kidney duck sera labeled with fluorescein isothiocyanate was injected intravenously into rabbits and its localization observed after intervals from one hour to several months. Animals killed after one hour showed an intense crisp fluorescence of the basement membrane of all glomeruli but no staining of any other part of the kidney or any other organ except for the spleen, where the substance appears irregularly in droplet form in the red pulp and in the lymph nodes. The fluorescein-labeled γ-globulin fraction of unmodified duck serum does not localize in the kidney, but it can be found in the spleen similar to the anti-rabbit-kidney duck serum. There is no unspecific "background staining" with this method. The labeled γ-globulin appears to be nontoxic and produces diseases just as well as anti-rabbit-kidney duck serum not conjugated with fluorescein.

This method seems to give a more quantitative impression of antibody localization than the original Coons method. Unspecific staining effects seem to be absent. The fact that a fluorescein-conjugated antibody against whole rabbit kidney localizes exclusively in the basement membrane seems to indicate that the basement membrane is the main, if not exclusive, antigenic factor in nephrotoxic sera.

It also points to the fact that under normal conditions the endothelium of the glomerulus is freely permeable to proteins of the size of γ-globulin, since the labeled antibody reaches the basement membrane without difficulty or delay. The persistence of the antibody during the later stages of experimental nephritis will be discussed in relation to the clinical and immunologic picture.

### 84. An Electrophoretic Method for the Detection of Leukocyte Antibodies

S. L. LEIKIN * and GLORIA D. ENG,*
Children's Hospital of the District of Columbia
Introduced by Robert H. Parrot

Specific antibodies against leukocytes have been produced in experimental animals. There is evidence to indicate that these are present in some clinical states, particularly certain leukopenias associated with hematologic disorders. However, difficulty has been encountered in the interpretation of leukagglutination tests because of false-positive clumping of leukocytes in control sera and the absence of positive reactions when they are expected.

It is known that protein antibodies which coat specific particles slow the electrophoretic mobility of these particles. This principle was used in developing a new method for determining the presence of leukocyte antibody.

Serum rich in anti-guinea-pig-leukocyte antibody was produced in rabbits. This was incubated with guinea pig leukocytes and then electrophoretic mobility determined. The electrophoretic velocity of the leukocytes exposed to the antibody-rich serum was demonstrated to be slower than that of the cells treated with rabbit serum not containing antibody.

This technique appears to be an accurate and rapid way of detecting the presence of leukocyte antibody. It has been used in the study of the relation of the antibodies of the ABO blood groups to leukocytes as well as in certain clinical conditions.

### 85. Effect of Hyperoxia and Hypercapnia on the Blood-Cerebrospinal Fluid Barrier

MIRIAM LENDING,*
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Introduced by A. Leonard Luhby

In our previous studies on hypoxia in puppies, there was increased permeability of the blood-cerebrospinal fluid barrier as measured by the rate of passage of albumin $I^{131}$ from blood to cerebrospinal fluid. Other investigators have shown alteration in vascular permeability with hyperoxia. The purpose of this study was to evaluate the effect of hyperoxia and hypercapnia on the blood-cerebrospinal fluid barrier in puppies.

One hundred per cent $O_2$ was administered by endotracheal intubation to puppies for periods of one to three hours immediately following the intravenous administration of albumin $I^{131}$ (11 μc. per kilogram). A control series of puppies was treated with a sham procedure. The experimental animals were divided in two groups. In the first group no attempt was made to prevent $CO_2$ accumulation, while in the second, a nonrebreathing valve was inserted to prevent $CO_2$ retention. Arterial $O_2$, pH, and $CO_2$ determinations were done in a sampling of animals in each group. Cisternal fluid and plasma specimens were obtained at the termination of the experiment in the control and hyperoxic animals and the radioactivity of each was measured in a well type scintillation counter. The ratio of cerebrospinal fluid radioactivity to plasma radioactivity was then calculated in each case.

The control series of puppies showed only minimal passage of albumin $I^{131}$ into the cerebrospinal fluid, the cerebrospinal fluid-plasma ratio was not greater than $60\times10^{-4}$. When $CO_2$ retention was allowed there was elevated blood $CO_2$ and decreased pH and the $O_2$ content ranged from 15 to

* By invitation.

18 vol. %, with $O_2$ saturation from 95 to 110 vol. %. These puppies showed increased rate of passage of $I^{131}$ into the cerebrospinal fluid, the cerebrospinal fluid-plasma ratios averaged $372 \times 10^{-6}$. When the nonrebreathing valve was used, the $O_2$ content ranged from 17 to 20 vol. %, with $O_2$ saturation from 109 to 116 vol. %, and there was normal blood $CO_2$ and pH. The cerebrospinal fluid-plasma ratios in these puppies were similar to those in the control series, indicating essentially no change in permeability of the blood-cerebrospinal fluid barrier.

A mixture of 7% $CO_2$, 72% $N_2$, and 21% $O_2$ was then administered to another group of puppies for periods varying from 30 minutes to 2 hours. The blood $CO_2$ was elevated and there was again a definite increase in the blood-cerebrospinal fluid barrier permeability as measured by the rate of passage of albumin $I^{131}$ from blood to cerebrospinal fluid.

Hypercapnia rather than hyperoxia would seem to be associated with increased permeability of the blood-cerebrospinal fluid barrier in puppies.

### 86. The Concentrating Defect in Sickle-Cell Disease

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A. DANIEL HAUSER,* and DEMETRA POLIMEROS,*
Mount Sinai, New York
Introduced by Horace L. Hodes

These studies were undertaken to attempt to explain the urinary concentrating defect in patients with sickle-cell disease. Six such subjects demonstrated normal renal hemodynamics. Maximum antidiuretic activity effected urine osmolalities between 300-500 mOs. per liter, with a mean of 440 mOs. A vasopressin (Pitressin) infusion (200 milliunits per hour) superimposed upon a 1,500 cc. water load promptly increased the urine osmolality from levels of 30-50 mOs. per liter to values commensurate with those produced by prior dehydration. In five hydropenic, vasopressin-infused subjects, GFR was reduced 50% by the intravenous administration of a ganglionic blocking agent and the application of venous tourniquets to the thighs. The fall in GFR, as in normal hydropenic subjects, produced a consistent fall in urine osmolality ranging from between 8%-20% and averaging 13%.

The concentrating defect is not attributable to any detectable alteration in renal hemodynamics or to a measurable solute diuresis. The prompt, albeit incomplete, response to ADH eliminates the possibility of absolute ADH unresponsiveness. The alternative to ADH-resistant impermeability to water in the distal or collecting tubule is weakened by the failure of the urine osmolality to rise as the rate of flow past these sites is reduced by a fall in GFR. By exclusion it therefore appears likely that sicklers are incapable of achieving an appropriate degree of medullary hypertonicity. These data, in conjunction with the morphological changes in the medulla described in sickle-cell subjects, tend to support the hypothesis that a normal vascular architecture at this site is prerequisite to the production of maximum medullary hypertonicity.

* By invitation.

### 87. Abnormal Inhibition of Lung Fibrinolytic Activity in Fibrocystic Disease of the Pancreas

JACK LIEBERMAN,* University of California
and Los Angeles County Harbor General Hospital
Introduced by Forrest H. Adams

The pathogenesis of the abnormally viscid mucus in fibrocystic disease has not as yet been determined. However, it appears that an abnormality in protein structure could be suspect, inasmuch as the viscosity of a solution is greatly affected by the character of its protein. To investigate the possibility of an abnormality in systemic proteolytic enzymes, the fibrinolytic system of lung and several other tissues was studied.

Since the tissue activator of plasminogen appears to have a localized function as compared to that of the other circulating fibrinolytic factors, primary attention was given to this activator in lung. Lung homogenates in saline were prepared from seven autopsied cases of fibrocystic disease. Activator activity was determined by a modification of the fibrin plate method of Astrup and Mullertz for the whole-lung homogenate, the supernatant fluid, the washed sediment, and a mixture of normal lung sediment plus the supernatant fluid or washed-sediment suspension of the fibrocystic specimens. Since the lung inhibitor is soluble, whereas the tissue activator remains in the sediment, the effect of the lung inhibitor can be differentiated from that of the lung activator of plasminogen. Simultaneous control studies were made on 95 autopsy specimens taken from fetuses, children, and adults dying from all sorts of inflammatory, neoplastic, vascular, and congenital processes.

Six of the seven cases of fibrocystic disease demonstrated no plasminogen-activator activity in the whole lung homogenates. The washed lung sediment, however, developed strong normal activator activity. In addition, the supernate from these six specimens completely inhibited normal lung plasminogen-activator activity. The seventh case appeared to have normal enzyme activity. Of the 95 control cases, only 2 showed a comparable pattern of intrinsic plasminogen-activator inhibition.

These data suggest that an abnormal inhibitor to the plasminogen activator exists in the lungs of children with fibrocystic disease. It can be postu-

lated that a defect in lung proteolytic activity may account for increased mucus viscosity through abnormal molecular configuration of the mucoprotein.

The pancreatic disturbance may also result from the same genetically determined enzyme abnormality. Trypsinogen may function in pancreas like the plasminogen activator does in lung, since trypsin is known to be an activator of plasminogen in addition to having its own proteolytic action. The marked similarity between lung plasminogen inhibitor and pancreatic trypsin inhibitor as described in certain species by Astrup may imply a single regulatory gene in these instances. The few reports claiming to have demonstrated trypsin in the stool or duodenal aspirate of an occasional patient with fibrocystic disease cannot be deemed valid since the substrates used were not specific for trypsin, but are similarly acted upon by chymotrypsin. A newborn infant included in this study who died of meconium ileus had absent intestinal trypsin but adequate levels of chymotrypsin within the pancreatic tissue.

It is postulated that fibrocystic disease is the result of a simultaneous inhibition of lung and pancreatic proteolytic activity which results in abnormally viscid mucus and its secondary obstructive features.

## 88. Poliovirus-Neutralizing Substances in the Feces of Paralytic-Poliomyelitis Patients

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It is very difficult or impossible to reinfect the intestinal tract of man or the chimpanzee with homologous poliovirus following infection or deliberate feeding of living virus. This has led to the search for specific neutralizing substances in the feces. Twenty-nine stools from 18 poliomyelitis patients were accordingly examined.

The following procedures were employed: Twenty per cent fecal suspensions were made with use of Hanks' BSS as suspending medium. The suspensions were centrifuged at 15,000 rpm for 30 minutes. The clear supernates were treated with penicillin and streptomycin, followed by heating at 56 C for one hour to inactivate living virus present in many of the preparations. Supernates were titrated against the three poliovirus types, with use of twofold dilutions from 1:2 to 1:64. One hundred $TCD_{50}$ of virus was added to the supernate dilutions. After incubation HeLa cells were added and the tubes incubated at 36 C for seven to nine days. End-points were recorded as the highest dilution of supernate neutralizing the added virus. Patients' serums were titrated in a similar manner, starting with a dilution of 1:4 to 1:8,192.

A case of paralytic poliomyelitis occurred in a 4-year-old child previously vaccinated with three injections of Salk vaccine appropriately spaced. A Type 1 poliovirus was recovered from stools collected five and six days after onset. Serial stool specimens were taken to determine the presence of antipoliomyelitis substance. Serum specimens were also taken at appropriate intervals. The following results of neutralization of Type 2 virus are expressed as the reciprocal of the highest dilution of stool or serum neutralizing added virus:

| Days after onset | 2 | 4, 5, & 7 | 24 |
|---|---|---|---|
| Stool titer against Type 1 | — | 32 | — |
| Serum titer against Type 1 | 1,024 | — | 8,192 |
| Serum titer against Type 2 | 256 | — | 256 |
| Serum titer against Type 3 | 512 | — | 2,048 |

Neutralizing substance for Type 1 or Type 3 virus was not demonstrable in the stools. The maximum serum titers were 1:4,096 against Type 1 virus; 1:4,096, for Type 2. Type 3 antibody was not demonstrable in the serum of this vaccinated child.

Specimens of other poliomyelitis patients stored at —20 C for four years were treated and titrated as above. Of 18 specimens from 17 cases, 3 extracts were shown to neutralize poliovirus. From a case in which Type 2 virus was previously recovered, neutralizing substance against the heterologous Type 3 was demonstrated. In two paralytic patients from whom no virus was recovered the fecal inhibitor was for the Type 1 virus only. In one of these cases the data as shown on the bottom of the page were obtained.

All stool specimens revealing the poliovirus-neutralizing substance gave positive reactions for human γ-globulin, but were all negative for human serum albumin in an immunological precipitin test employing appropriate rabbit antisera, thus indicating the selective presence of γ-globulin in intestinal excretions. The type specificity and thermostability of this substance suggests its antibody nature.

* By invitation.

| Days after onset | 5 | 12 | 15 | 18 | 21 | 26 | 27 | 40 | 41 | 44 | 48 | 51 | 56 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stool titer | <2 | — | 4 | — | 16 | 16 | — | 4 | 8 | 16 | 16 | 8 | 8 |
| Serum titer | 1,024 | 4,096 | — | 4,096 | 2,048 | — | 2,048 | — | — | — | — | — | — |

### 89. Nitrogen-Balance Studies in Premature Infants Fed ad Libitum

LULA O. LUBCHENCO, University of Colorado

Twelve infants, six boys and six girls, with birth weights of 1,050 to 1,370 gm. were studied for nitrogen and electrolyte balance starting from the first to the eighth day after birth and continuing until discharge from the nursery at 7 to 13 weeks. Six of these infants were permitted ad libitum ("demand") feedings, and six were given "scheduled" feedings not to exceed 120 Cal. per kilogram per day. Nitrogen, sodium, and potassium intakes were measured, and the excretion of nitrogen, creatine, creatinine, sodium, and potassium were determined in the urine and stool. Serum electrolyte determinations were performed weekly.

The caloric intakes during the first five weeks after birth of both "scheduled" and "demand" fed babies were similar. During the 6th to 13th weeks, four of the infants on "demand" ingested an average of 164 Cal. per kilogram per day for a total of 15 weeks. The other two infants on "demand" were satisfied with less than 130 Cal. per kilogram per day.

The weight gains of the four infants fed ad libitum who were taking a high-caloric diet were compared with the six "scheduled" infants and the two infants on "demand" who ingested smaller amounts, and it was noted that the increased caloric intake did not result in an increased weight gain.

When nitrogen retention was compared with nitrogen intake, a significant correlation was found. The relationship of increased retention with increased intake existed up to the maximum intake of 1,300 mg. per kilogram per day of nitrogen. The per cent of ingested nitrogen which was retained varied with age in both the infants fed by "schedule" and by "demand." Median retentions for the group reached a peak of 64% at four weeks after birth and gradually decreased to 50% at nine weeks.

Since premature infants with the higher nitrogen retentions did not gain more weight, it would be expected that they would show additional evidence of tissue production, such as increased creatinine excretion and potassium retention. The creatinine excretion of the four "demand" fed infants during the time they were taking more than 130 Cal. per kilogram per day was significantly higher than the other eight babies during the same period of nursery stay. The average creatinine excretion of the four "demand" fed infants was 15 mg. per kilogram per day, in contrast to 11 mg. for the other eight.

Potassium retention values increased with nitrogen retention and was greater than 3.2 mEq. per gram of nitrogen. Sodium was more labile in its retention than potassium, and there was a sharp decrease in sodium retention during the last two to three weeks of nursery stay in most of the infants.

These data support the findings of others that premature infants who ingest large amounts of protein also retain large amounts of nitrogen and, in addition, they indicate that the increased retention does not result in an increased weight gain, but rather in a different body composition.

### 90. The Relation of the Serum Tocopherol Level to Nitrogen Retention and to the Urinary Excretion of Creatine, Creatinine, and Amino Acids in Premature Infants

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University of Colorado

In experimental animals a relationship between low serum tocopherol levels and increased creatinuria, amino-aciduria, and decreased nitrogen retention has been found. Premature infants who are fed on a low-fat diet develop low serum tocopherols. They also show a significant amino-aciduria. Because the creatinuria in premature infants, although slight, is increased in infants fed a high-protein diet and increases with the amount of nitrogen excreted, studies have been carried out to investigate the role of tocopherol in the nitrogen metabolism of premature infants.

Nitrogen balance studies were performed on 10 infants, ages 35-75 days, whose birth weights were less than 1,500 gm. These were compared with 12 infants of similar ages and weights supplemented with tocopherol from birth. Serum tocopherol, nitrogen retention, and urinary excretion of creatine, creatinine, and amino acids were measured. The infants were fed a partially skimmed milk mixture (Dalactum) ad libitum. On this feeding schedule their intake of protein was 4-6 gm. per kilogram per day. Urine and stool collections were made for five days before and five days during the daily oral administration of 10-20 mg. of $\alpha$-tocopherol acetate emulsion. In three infants, collections were carried out for a subsequent five-day period.

The initial serum tocopherol levels varied from 0.08 mg. % and rose after supplementation to 0.23-3.85 mg. %.

The 10 infants who received no tocopherol supplements excreted an average of 1.4 mg. of creatine per kilogram per day as compared to the control infants who excreted an average of 2.7 mg.

In 5 of 10 infants, there was a decrease in the degree of creatinuria following oral administration of $\alpha$-tocopherol, and in 5 infants there was an increase in creatinuria.

Creatinine excretion averaged 11.0 mg. per kilogram per day before supplementation, 12.2 mg. after supplementation, and 13.9 mg. in the control

* By invitation.

group. Nitrogen retention was 458 mg. per kilogram per day before supplementation, 449 mg. after supplementation, and 470 mg. in the control infants. No change in amino-aciduria occurred with oral administration of tocopherol and ranged from 13-22 mg. before and 14-17 mg. during tocopherol supplementation.

Our studies indicate that nitrogen retention, creatinine excretion, and amino acid excretion were not altered by the addition of $\alpha$-tocopherol to the diet. The effect of $\alpha$-tocopherol on creatine excretion could not be defined.

### 91. New Concept Regarding Maternal Cyanocobalamin (Vitamin $B_{12}$) Available for Placental Transfer

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The cyanocobalamin (vitamin $B_{12}$) level of the serum of pregnant women during the last trimester is low, approaching those found in pernicious anemia, whereas the fetal cord blood levels are high. This phenomenon has been explained as being due to fetal parasitization of the mother. Our studies employing radioactive cyanocobalamin as a physiological tracer in dogs and humans have shown that the low maternal cyanocobalamin serum level may be due to (*a*) lowered absorption of orally administered cyanocobalamin during the last trimester of pregnancy, (*b*) competition between mother's tissue and the fetus for the cyanocobalamin in the maternal blood. The latter, for the most part, appears to be dependent upon the mother's intake of this vitamin.

Additional studies which support our interpretation are as follows:

Three women who had been given small doses of $Co^{58}$-cyanocobalamin two weeks prior to delivery during previous experimental periods subsequently became pregnant again. At the time of second delivery, the radioactivity retained over the hepatic area was low, being 140, 125, and 100 counts per minute above background, respectively. No radioactivity was detectable over the hepatic area of the second infants. The placentas were also found to be free of radioactivity. It was reasoned that the residual $Co^{58}$-cyanocobalamin in the women was of such low magnitude that the proportion to be placentally transferred was undetectable in the fetus or that the maternal liver and tissue stores of cyanocobalamin were tightly bound and essentially unavailable for placental transfer.

To determine which of the above mechanisms was operative, a pregnant woman, two weeks before delivery, was given a small, 0.057$\mu$c., oral dose of $Co^{58}$-cyanocobalamin, calculated to result in a low hepatic area count comparable to that of the previous subjects prior to their second delivery. This dose resulted in 90 counts per minute above background. After delivery, the hepatic area of the infant had a radioactivity of 200 counts per minute above background, indicating considerable placental transfer of $Co^{58}$-cyanocobalamin.

It is apparent from these experiments that "labile" and "stable" stores of cyanocobalamin exist in the pregnant woman. The "stable" stores of the tissues, of which the cyanocobalamin in the liver is representative, is probably not ordinarily available for placental transfer, as is illustrated by the first three women who inadvertently received their radioactive cyanocobalamin prior to conception. Their infants contained no measurable radioactivity. On the other hand, the blood cyanocobalamin reflects the "labile" stores of this vitamin and may be the main source of fetal cyanocobalamin, unless perhaps maternal dietary deprivation occurs. This "labile" store appears to be highly dependent upon the mother's dietary intake of cyanocobalamin.

### 92. Additional Observations Concerning the Defect in an Infant with Hypoglycemia Precipitated by Leucine

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In 1956, Cochrane et al. (*J. Clin. Invest.* 35:411, 1956) first reported that administration of L-leucine precipitated hypoglycemia in some infants with "idiopathic" hypoglycemia. Since isovaleric acid, one of the metabolites of leucine degradation, produced hypoglycemia in the one affected patient tested, it was suggested that the defect might be located at a point further in the degradation pathway. In our patient, a leucine-sensitive infant, isovaleric acid failed to produce hypoglycemia; however, $\alpha$-ketoisocaproic acid, the intermediate metabolite between leucine and isovaleric acid, did produce marked hypoglycemia.

The affected infant is now 9 months of age, and his course to date has been characterized by hypoglycemia and seizures since birth and developmental retardation. The chronic hypoglycemia has been partially ameliorated by the administration of corticotropin and by the use of a formula (Nutramigen) containing approximately half the amount of leucine contained in cow's milk.

The infant reacted to standard test doses of glucose by exhibiting an elevation in blood sugar to only 100-106 mg. % at one hour and a fall to 21-30 mg. % at two to three hours, at which time seizures usually occurred. Epinephrine and glucagon elicited initial moderate hyperglycemic responses, with a rapid decline to hypoglycemic levels.

The blood sugar was 23 mg. % 40 minutes after oral feeding of casein (1.5 gm. per kilogram) and

* By invitation.

13 mg. % 30 minutes after oral administration of L-leucine (0.15 gm. per kilogram). L-Leucine (75 mg. per kilogram), injected intravenously, precipitated profound hypoglycemia on repeated occasions, 25-30 minutes after injection; this did not occur in seven other patients, two of whom also had hypoglycemia. Isovaleric acid was administered orally in doses which were two to five times as large as those used by Cochrane but failed to induce hypoglycemia. However, the intravenous administration of α-ketoisocaproic acid resulted in profound hypoglycemia after 15 to 20 minutes. Paper chromatography for amino-aciduria and qualitative examination of urine specimens (not during loading tests) for α-ketoisocaproic acid were negative.

Leucine administered to the parents did not produce hypoglycemia in the mother but did to a moderate degree in the father, whom we plan to study further.

These studies suggest that the defect in this patient is in the conversion of α-ketoisocaproic acid to isovaleric acid (leucine ⟶ α-ketoisocaproic acid ⟶ isovaleric acid ⟶ acetoacetic acid).

The intravenous leucine-tolerance test used as a diagnostic aid is described. The relationship of these findings to those reported by Cochrane and the possible relationship of the defect observed in this patient to that seen in so-called maple syrup disease will be discussed.

### 93. An Early Inflammatory Skin Lesion Induced in Guinea Pigs with PPD

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University of Pittsburgh
Introduced by Stuart S. Stevenson

It has long been held that the heating process during the manufacture of tuberculin purified protein derivative (PPD), caused sufficient denaturation of the tuberculoprotein to abolish most of its biological properties. Yet PPD does retain the specific ability to elicit the tuberculin reaction. Sera from rabbits that received repeated injections of phenol-killed human tubercle bacilli, Strain H37 Rv, do not precipitate with PPD (Boyden). Although PPD is a poor antigen, it will precipitate with rabbit antisera made by immunizations with PPD or unheated tuberculoprotein (Seibert). Previous experiments in this laboratory suggested that PPD does not precipitate with guinea pig antisera produced similarly.

As the Arthus reaction depends upon precipitating antibodies against the specific antigen (Kabat), a histological study was undertaken on the four-hour skin reactions observed in actively immunized guinea pigs with PPD or unheated tuberculoprotein. The skin lesions elicited with PPD and unheated tuberculoprotein in the two groups of guinea pigs were similar both grossly and histologically to mild Arthus reactions. However, 100μg. of PPD were necessary to produce the same sized lesions as 10μg. of unheated tuberculoprotein.

Sera from these guinea pigs were studied by the Preer agar-diffusion method for the presence of precipitating antibodies. Positive results were obtained only when unheated tuberculoprotein was added to the sera from animals that were immunized with unheated tuberculoprotein. Whether the observed PPD skin reaction is one incidence of an Arthus reaction occurring without the presence of precipitating antibodies or if there was an insufficient quantity of precipitating antibodies to be measured by the Preer method is currently being investigated.

### 94. The Effect of Synthetic Angiotonin upon the Systemic, Pulmonary, and Coronary Hemodynamics and Myocardial Metabolism of the Intact Dog

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J. E. CLIFFORD,* C. W. CRUMPTON,* and
G. G. ROWE,* University of Wisconsin
Introduced by Nathan J. Smith

Angiotonin has been related to renal hypertension, and the present study was designed to show some facets of the action of this substance in the intact dog.

*Material and Methods.*—Mongrel dogs, weighing 15-25 kg., were anesthetized with morphine (3 mg. per kilogram subcutaneously), followed in one hour by Dial-methane-Nembutal (50:50 mixture 0.25 ml. per kilogram intravenously). Cardiac output (Fick), and coronary blood flow (nitrous oxide, Fick), were measured before and after the infusion into the right atrium of synthetic angiotonin octapeptide (Courtesy of Ciba Corporation, Basle); 0.3 mg. was given over the experimental period of 25 minutes. Left ventricular weight was measured after killing.

*Results.*—Significance was established at 5% level or less.

General metabolism and respiratory exchange were unaffected. Rate and cardiac output did not vary significantly from control values. Mean femoral arterial pressure increased from 113 to 148 mm. Hg ($<0.01$). Left ventricular work increased, as did calculated peripheral resistance (from 4,180 to 6,045 dynes).

Coronary blood flow was maintained at control values, but coronary vascular resistance increased ($P<0.05$). The oxygen content of the coronary sinus blood decreased from 4.8 to 2.8 vol. % ($P<0.05$), and myocardial oxygen extraction increased from 11.7 to 14.5 vol. %. The oxygen consumption of the left ventricle increased sig-

* By invitation.

nificantly, whether measured directly or as a cardiac metabolic rate. Similar trends for cardiac carbon dioxide metabolism were observed. Cardiac efficiency, measured by index value, or directly, showed a slight decrease.

Angiotonin then increases systemic and coronary vascular resistance without, however, decreasing coronary blood flow. Left ventricular work is increased, and this may relate partially to the increased cardiac oxygen consumption.

### 95. Neuronal Necrosis and the Phrenischemic Episode

A. JAMES MCADAMS,* NOBORU KOBAYASHI,* and BENJAMIN H. LANDING, Cincinnati

A comparative study of the cellular and distributional changes of "anoxic" brain damage has been made in 12 selected cases of infants and children. The criterion for inclusion was the occurrence of selective neuronal necrosis (Scholz). The cases were placed in three groups according to their clinical picture: Group 1, three cases with perinatal anoxia; Group 2, two cases with cardiac standstill and two cases with cardiovascular collapse; Group 3, two cases with prolonged convulsions and three cases with primary encephalitis.

The hematoxylin and eosin appearance of the necrobiotic neuron is distinctive and especially characterized by a bright eosinophilic appearance to the cytoplasm. Histochemically, the outstanding properties of such cells are increased acidophilia and basophilia, sudanophilia, and periodic acid-Schiff-positivity, which is resistant to pyridine extraction. These and other properties suggest the accumulation of a new substance(s) in the cytoplasm, histochemically similar to ganglioside.

The selective nature of neuronal necrosis, in the situations studied, has been confirmed and extended. The general pattern consists of greater severity in the depths of sulci, greater severity in the dorsum of the hemispheres with relative sparing of the poles, hippocampal involvement which is minimally expressed as Sommer's sector necrosis, especial tendency to involvement of the globus pallidus and medial part of thalamus, and Purkinje-cell involvement.

The mechanism of selective neuronal necrosis is widely accepted as one of regional susceptibility, mediated by vascular spasm. Proof of vascular spasm, or even the capability, however, is lacking. The concept of regional (topistic of Scholz) susceptibility, moreover, demands acceptance of a complexity of metabolic organization within the central nervous system that is probably unreasonable.

The patterns of distribution of selective neuronal necrosis are readily explained on the basis of the vascular architecture of the brain in that compression of vessels secondary to edema can account for most, if not all, of the features. Analysis of our Group 2 patients (cardiac arrest and severe shock) provides an adequate explanation for the edema. It is maintained that failure of brain perfusion leads to the rapid accumulation of metabolic products, acid in their reaction, which greatly alter vascular permeability. This is descriptively stated as the phrenischemic episode.

The likelihood of a similar mechansim exists for the cases of the other groups. An apparent relative sparing of the immature cerebral cortex in the Group 1 cases offers a possible explanation for certain instances of cerebral palsy in which there are severe motor defects in the absence of demonstrable mental deficit. The relative limitation of involvement to the cortex in three cases of encephalitis suggests that preexisting edema imposes a liability to neuronal necrosis.

### 96. The Content of Hageman Factor in the Blood of Newborn Infants

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Introduced by Angelo DiGeorge

Ratnoff has described, and others have confirmed, the existence of the Hageman factor, a thromboplastin component which is concerned with the surface activation of coagulation. Although this factor is apparently without clinical significance, it is of considerable theoretic interest. The origin of the Hageman factor is obscure. In an effort to correlate this factor with other coagulation factors, particularly prothrombin, Factor VII, and plasma thromboplastin component (PTC), the concentration of Hageman factor has been measured in the blood of full-term infants during their first week of life.

The mean content of Hageman factor is decreased in newborn infants when compared to adult normal levels. However, there is considerable scatter with the levels, varying from 25% to 100% of adult normal. The mean levels rise to nearly normal by the fourth day of life without regard to vitamin K administration. There is no parallelism between the levels of Hageman factor and those of PTC and prothrombin.

### 97. Treatment of Terminal Leukemic Relapse by Total-Body X-Irradiation and Intravenous Infusion of Autologous Bone Marrow Stored During Remission

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Massachusetts General Hospital
Introduced by Nathan B. Talbot

During a remission of acute leukemia in children induced by one of the chemotherapeutic agents

* By invitation.

the bone marrow may return temporarily to a normal morphologic state. Thus the question arises whether in some instances the marrow may provide an acceptable source of autologous material for subsequent use as a marrow autograft. It has been reported that the infusion of autologous marrow in animals and isologous marrow in man after potentially lethal doses of radiation or chemotherapeutic agents can protect against their otherwise lethal effects by restoring the marrow-cell population. It has also been shown that marrow may be preserved for long periods in glycerol at −70 C and still retain the viability essential for its eventual use as an inoculum for the repopulation of denuded marrow spaces. An investigation of the effects of preserved autologous marrow in patients with leukemia, along with parallel studies in dogs, was carried out. The purpose of this communication is to report early results in three patients of the administration of preserved autologous marrow that was taken during a period of remission from leukemia and subsequently infused after the patient had received a potentially lethal dose of total-body x-irradiation in a period of critical relapse.

One case died 9 days after irradiation, with leukemia still present; a second case died 23 days after irradiation, with no evidence of leukemia or repopulation of the bone marrow. A third case showed recovery of normal marrow within 17 days after irradiation (Dec. 6, 1958) and had a clinical and hematologic remission lasting to date. The rapidity of the recovery of normal bone marrow and peripheral blood after radiation exposure in the potentially lethal range suggests that a successful marrow autograft was the cause of this patient's improvement. These findings would seem to indicate that further investigation of this mode of therapy in leukemia is warranted.

### 98. The Urinary Excretion of Nonvolatile Ether-Soluble Acids in Children

WILLIAM J. MELLMAN,* LINDA BOCKRIS,* and LEWIS A. BARNESS, University of Pennsylvania

Normal humans excrete various organic acids, including amino acids, phenolic acids, fatty acids, and acids of the Krebs cycle. Ether-soluble acids include chiefly the acids of the Krebs cycle plus lactic acid, pyruvic acid, propionic acid, and hippuric acid. By drying the ether-soluble acids under reduced pressure, the volatile acids are removed.

Di-carboxylic acid excretion in well children and in children with various diseases were studied. Six patients in the group studied were found to have abnormally high excretions (over 0.65 mEq. per kilogram per 24 hours). Three of the abnormal group suffered from congenital glaucoma, albuminuria, acidosis, and neuromuscular retardation. The others included one with methylsalicylate poisoning, one with renal tubular acidosis (de Toni-Fanconi-Debre type), and one with cystic fibrosis of the pancreas.

Ether-soluble acid titrations correlate well in the normal with creatinine excretion. Total $\alpha$-amino acid concentrations in this group of patients was found to range from 0.22 to 2.47 mg. nitrogen per kilogram per 24 hours, well within the normal limits. There was no significant association between $\alpha$-amino nitrogen excretion and ether-soluble acid excretion. The former showed the expected response to protein intake while ether-soluble acid excretion did not show the same sensitivity to dietary protein.

The urines were chromatographed by two systems, with use of both ether extracts and eluates of urine placed directly on anion-exchange columns. Several of the organic acids have been identified.

Critic, succinic, and hippuric acid are consistently present in normal and abnormal urines. Other Krebs-cycle intermediates were found in large amounts in the patient with methylsalicylate ingestion. Two of the patients with congenital glaucoma excreted excessive amounts of an acid with characteristics of methylmalonate (*J. Biol. Chem.* 221:93, 1956). This acid has been found to be present in large quantities in urine of rats who develop liver necrosis on a yeast diet and in small amounts in normal human urines.

### 99. Glucose-6-Phosphate Dehydrogenase Activity in Patients with Various Abnormal Hemoglobins

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The high incidence of sickle-cell anemia and glucose-6-phosphate dehydrogenase deficiency in populations originating from Africa and the Near East makes it important to describe the pattern of clinical disease in persons with both defects and to investigate the possibility of gene interaction in the two conditions. The present report is based upon the study of 106 persons with various abnormal hemoglobins, of whom 75 were homozygous for hemoglobin S, 10 had sickle-cell hemoglobin C disease, and 21 had sickle-cell trait. A similar group of normal controls was also studied. In each instance the glucose-6-phosphate dehydrogenase activity in the red cells was determined by the method of Zinkham and the glutathione stability test by the method of Beutler.

Eight persons from five families were found to have homozygous sickle-cell disease combined with glutathione instability and low levels of glucose-6-

* By invitation.

phosphate dehydrogenase. In addition, three persons from three different families with sickle-cell hemoglobin C disease and two in two different families with sickle trait were found to have the afore-mentioned combined hemoglobin-enzyme abnormality. Contrary to the expectation that persons with the combined defect might demonstrate a severer clinical course, it was found that their clinical manifestations were no worse than those of similar persons with normal enzyme levels. In addition, in all instances the two defects segregated independently of each other.

In the course of these studies it was found that the mean glucose-6-phosphate dehydrogenase among patients with homozygous sickle-cell anemia was 347.0 ±94.9 units/100 ml. of RBC, while that of the normal controls was 220.7 ±64.3 units/100 ml. of RBC. This difference, which is statistically significant, is thought to result from the younger mean cell age of the red cell population which is characteristic of sickle-cell anemia. Erythrocyte survival studies with use of $Cr^{51}$ are currently in progress.

### 100. Observations on Amino-Aciduria in the Premature Infant

DONOUGH O'BRIEN,* University of Colorado
Introduced by Lula O. Lubchenco

Examination of serial 24-hour urine specimens from a group of small premature infants, aged 1 to 8 weeks (birth weights 1,020 to 1,410 gm.), has yielded further information on the amino-aciduria of this age group. Eleven of these premature infants, fed on a half-skimmed milk and carbohydrate mixture (Dalactum), were subjected to continuous urine collections for the first eight weeks of life. Aliquots from the 24-hour sample, collected on the fourth day of life and from subsequent weekly pools thereafter, were stored in the freezer until they were assayed for total nitrogen by the Kjeldahl method, for free α-amino nitrogen by the copper method after elution from a Dowex 50 column, and for creatinine by the Jaffe reaction after ether inactivation of interfering chromogens. The mean values for urinary amino acid excretion are shown in the tabulation.

Individual amino acid components were identified by two-dimensional chromatography, with use of both a phenol-butanol acetic acid system and a methanol, tertiary butanol-methylethylketone, diethylamine system and estimated visually against a series of standards.

The results show a progressive increase in the amino nitrogen excretion during the first four weeks of life with a slower rise thereafter. A significant correlation was found between the amino nitrogen and the total nitrogen and between the amino nitrogen and the creatinine in the urine.

Among the individual amino acids, the greatest urine concentrations were those of glycine and proline, and these rose sharply throughout the assay period. An intermediary group consisting of histidine, threonine, alanine, methyl glycine, serine, tyrosine, and glutamic acid showed a sharp increase in the rate of excretion until the third week, with a subsequent leveling off. In contrast, glutamine, tryptophan, cystine, leucine, isoleucine, aspartic acid, and valine showed only a small increase in the urinary excretion in the first two

* By invitation.

| | Week | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII |
| Total α-Amino Nitrogen | 371* | 678 | 700 | 1,028 | 971 | 1,128 | 1,142 | 1,121 |
| Glycine | 94 | 114 | 152 | 156 | 196 | 200 | 136 | 205 |
| Alanine | 17 | 35 | 68 | 74 | 65 | 76 | 81 | 95 |
| Glutamine | 22 | 19 | 23 | 18 | 13 | 33 | 13 | 11 |
| Serine | 42 | 36 | 44 | 46 | 60 | 55 | 66 | 94 |
| Threonine | 18 | 31 | 65 | 71 | 59 | 77 | 65 | 81 |
| Glutamic acid | 7 | 31 | 48 | 41 | 48 | 48 | 57 | 55 |
| Histidine | 21 | 30 | 56 | 68 | 77 | 79 | 84 | 85 |
| Tyrosine | 13 | 30 | 38 | 40 | 49 | 49 | 60 | 66 |
| Tryptophan | 2 | 12 | 25 | 13 | 24 | 20 | 27 | 26 |
| Aspartic | 13 | 15 | 14 | 28 | 12 | 24 | 11 | 21 |
| Valine | 2 | 9 | 9 | 11 | 10 | 12 | 17 | 18 |
| Leucine & isoleucine | 6 | 18 | 34 | 23 | 28 | 32 | 33 | 30 |
| Proline | 21 | 50 | 90 | 83 | 110 | 133 | 148 | 136 |
| Methyl glycine | 9 | 22 | 57 | 55 | 74 | 75 | 90 | 81 |
| Hydroxyproline | 12 | 22 | 31 | 33 | 46 | 43 | 43 | 29 |
| Cystine | 7 | 7 | 7 | 9 | 8 | 15 | 8 | 9 |

* Micromols per kilogram per 24 hours.

weeks, thereafter maintaining a relatively low level of excretion. The excretion of hydroxyproline, both in rate of increase and in total amount, was intermediate between the last two groups.

Despite the general trends of the means, the range in the excretion of individual amino acids was great, and it was difficult to discern any constant progression of the amino acid pattern.

The excretion of large amounts of hydroxyproline and of methyl glycine and the relatively low urine content of glutamine in relation to glutamic acid, as compared with older children, is noted.

The techniques will be described and the significance of the findings discussed.

### 101. Protein Binding of Bilirubin and Its Possible Relationship to Kernicterus

GERARD B. ODELL,* Johns Hopkins
Introduced by Robert E. Cooke

In a previous study it was demonstrated that bilirubin bound to albumin could be dissociated by certain sulfonamides and salicylate. The amount of bilirubin dissociated by a given concentration of salicylate or sulfonamide was directly proportional to the relative saturation of the albumin with bilirubin, and the dissociated bilirubin was free to diffuse through semipermeable membranes.

In the present report the saturation of serum proteins with bilirubin was studied in the sera from 40 infants with neonatal jaundice of various causes. The saturation was measured by determining the amount of protein-bound bilirubin which could be dissociated by added salicylate.

The serum samples was diluted with an isotonic phosphate buffer, pH 7.4; 2.5 ml. of the diluted sample was placed in each of two cuvettes and the spectral absorption curve measured in a Beckman spectrophotometer. The phosphate buffer was used as a blank. The protein-bound bilirubin was determined from the corrected spectral absorption of the samples at 460 mμ before and after the addition of salicylate and compared to the control sample, which was diluted with an equal volume of buffer. The difference in protein-bound bilirubin in the two samples was expressed as the percentage change from the total protein-bound bilirubin and was used as an index of the relative saturation of the serum proteins with bilirubin. The results indicated that the degree of protein saturation with bilirubin was directly proportional to the total bilirubin concentration and inversely proportional to the serum protein concentration. In addition, it was found that the serum proteins were saturated with bilirubin at lower concentrations if the serum contained heme pigments which absorb from 405-415 mμ.

One infant with erythroblastosis who died because of a transfusion accident was found at postmortem examination to show microscopic evidence of early mild kernicterus. This infant had one of the highest bilirubin saturations of his serum proteins that we have found by the above technique, although his bilirubin concentration was only 21 mg. %. The remaining 39 infants have survived and had no obvious signs of neurological damage at the time of their hospital discharge.

These studies emphasize that total bilirubin concentration is only one variable of many which alter the diffusion gradient of bilirubin from extracellular to intracellular fluids. Heme pigments, sulfonamides, and salicylate increase diffusible bilirubin, whereas serum albumin will reduce the diffusible bilirubin and thereby can change the direction of the diffusion gradient.

### 102. Early Administration of Sodium Bicarbonate to Young Children with Acute Salicylism

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Introduced by Warren E. Wheeler

Sodium bicarbonate is considered to be contraindicated in the treatment of acute salicylism despite the logic of hastening the reduction of serum salicylate by increasing the urinary excretion of free salicylate. The reason for this belief is the demonstration in older children and adults of a respiratory alkalosis following salicylate ingestion; a farther rise in serum pH incurs the risk of tetany or convulsions. Although there is reason to believe the acid-base patterns of infants and toddlers following the acute ingestion of salicylates is different from adults, studies of the effect of $NaHCO_3$ in this age group are lacking.

In the last year 39 children ranging in age from 14-18 months have been studied following the accidental ingestion of salicylates. All have had serial measurements of serum salicylate. For purpose of analysis the children have been divided into three equal groups. A control group received a water load in excess of 3.0 liters per square meter per 24 hours. The second group, in addition to a similar intravenous fluid load, were given $NaHCO_3$ I. V. over a two- to four-hour period shortly after fluid therapy was begun in a dosage calculated to raise the serum $CO_2$ content 5-7 mEq. per liter. A third group received fluids in amounts less than 2.0 liters per square meter per 24 hours.

The children were seen 1-22 hours (mean 4.8) following ingestion at which time the mean salicylate level was 41.5 mg/100 ml. (range 17-76.5). The initial acid-base pattern was that of a mixed respiratory alkalosis and metabolic acidosis: mean pH 7.36 (7.07-7.50), $CO_2$ 18.2 mEq. per liter (3.6-21.9), $pCO_2$ 31.1 mm. Hg (11.6-39.4). In only three cases was the pH elevated

* By invitation.

beyond 7.43, the values being 7.44, 7.46, and 7.50. Thus, in contrast to adults, uncomplicated respiratory alkalosis occurs infrequently in young children with acute salicylism and is of mild degree even then.

In two of the subjects receiving $NaHCO_3$, alkalinization of the urine to values greater than pH 6.5 was not achieved and the rate of fall of serum salicylate did not differ from the control group. In the remaining 11 children the fall in serum salicylate was significantly faster than the control group. Eight hours after the onset of therapy in the control patients none had serum salicylate values less than 15 mg/100 ml. and the mean salicylate value had fallen only 33% from the pretreatment level. In contrast, 8 of 11 patients treated with bicarbonate had values less than 15 mg/100 ml. at eight hours, at which time the mean level had fallen 52% from the initial level. Quantitative urine analyses demonstrated the marked salicyluria following alkalinization of the urine.

The acid base effects of bicarbonate loading were modest and transient. The values observed were pH 7.49 (7.35-7.64), $CO_2$ 25.4 mEq. per liter (20.1-29.5), $pCO_2$ 32.8 mm. Hg (20.1-39.0). No untoward effects of bicarbonate administration were noted. Transient hypokalemia occurred in one subject. Symptoms of salicylism abated more rapidly in this group than in the controls.

In eight subjects receiving less than 2.0 liters per square meter per 24 hours serial salicylate levels were performed beyond 16 hours. The fall in serum salicylate in this group did not differ significantly from the control group.

The early administration of sodium bicarbonate in the dosage described to young children with acute salicylism is both efficacious and safe. Its use is recommended.

### 103. Remission in Childhood Nephrosis: Report of Data Demonstrating the Essential Role of Renal Tubular Function

WILLIAM J. OLIVER,* Ann Arbor
Introduced by James L. Wilson

The means by which steroid therapy induces a remission in nephrosis is not known. The present study has investigated the sequence of events occurring prior to, during, and immediately following steroid-induced diuresis in childhood nephrosis.

Current hypotheses suggest that the edematous state of childhood nephrosis follows primarily from the glomerular leakage of serum proteins (with resultant decreases in serum oncotic pressure and plasma volume, accompanied by compensatory increases in aldosterone and antidiuretic hormone activity). The glomerular lesion is thus implicated as the major site of pathology. Reversal of the active, edematous phase of nephrosis might then be expected to follow approximately the same pattern of events. However, the observed data to be reported do not fit the above hypothesis but instead demonstrate the occurrence of a distinct alteration in renal tubular function which is essential for the observed diuresis.

The observed chronology of events may be summarized:

A. During active nephrosis, with edema, serum oncotic pressure is extremely low (50-90 mm. of water pressure, compared with the normal range of 330-400 mm. of water pressure). Concurrent studies of serially collected urines show a large constant loss of potassium while sodium is avidly retained producing urinary Na/K ratios in the range of 0.02:0.10. Further retention of sodium occurs when steroid therapy is initially begun.

B. After institution of steroid therapy, the above findings persist essentially unchanged, until after a varying time has elapsed, then diuresis can be anticipated by two events: 1. Urinary sodium concentration and total daily excretion increase, accompanied by or followed in 24 to 48 hours by increased volume of urine. 2. Significant decreases in urinary protein usually, but not always, occur. Serum oncotic pressure, however, remains essentially at prediuresis levels until after diuresis has become well established, then slowly returns to normal many weeks later. On the other hand, serial plasma volumes show no significant change from prediuresis values. Salivary Na/K ratios parallel the observed urinary Na/K ratios. Antidiuretic hormone activity, as indicated by urinary osmolarity, does not become depressed.

In summary, the observed data demonstrate a distinct pattern occurring during remission of childhood nephrosis which cannot be fitted to previous concepts depicting the pathophysiology of the active disease. Although alterations in glomerular premeability occur with remission, the reversal of renal tubular activity in respect to sodium transport would appear to occur as an essential event in the resulting diuresis. The exact manner by which steroid therapy produces these events lends itself to numerous speculations, especially in regard to the role of "glomerular-tubular imbalance" in the transition from the edematous to nonedematous phase of nephrosis. Further studies upon this aspect are in progress.

### 104. The Carbon Dioxide Spirometer: A New Instrument for the Rapid Evaluation of the Circulation

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Respiratory air flow is routinely measured by recording the differential pressure across a low-resistance screen while a subject breathes through

* By invitation.

it. Respiratory carbon dioxide concentration can be accurately recorded by sampling continuously through a rapid-response infrared detector. If the respiratory flow curve is continuously multiplied by the carbon dioxide concentration curve (with a suitable delay circuit to bring the two exactly into phase), the resulting curve represents carbon dioxide flow. Both these flow curves can be simultaneously electrically integrated into volume curves. The carbon dioxide spirometer consists of suitable sensing devices, an eight-channel recorder, and an analogue computer with an adjustable time delay with suitable multiplying and integrating circuits, to perform these operations and record the results. When a patient breathes through its mouthpiece, the resulting record contains the curves which would be written on an ordinary spirometer plus those which would be written on a spirometer which collected carbon dioxide only. In addition to flows and volumes of both air and carbon dioxide, it can record carbon dioxide vital capacity, single-breath carbon dioxide excretion curves, etc.

This instrument allows the easy and accurate measurement of transit time of metabolite (carbon dioxide) from muscle cell to lung. By subtraction of the blood circulation time, this allows estimation of the interstitial transit time. Examples will be shown of the rapid appearance and elimination of carbon dioxide after a short period of exercise in healthy children, contrasted with its slow appearance and slow excretion in children with heart disease. For instance, the inability of the patient with pulmonic or aortic stenosis to increase his cardiac output adequately on exercise shows up clearly as a long low curve of $CO_2$ excretion after short exercise. The special physiological problems faced by the patient with a large left-to-right shunt will be demonstrated. These patients, after exercise, hover on the edge of severe arterial alkalosis, unable to make full use of their relatively normal pulmonary function. Preliminary data on the early diagnosis of cardiac failure will be presented, as well as tentative data on the differentiation of patients in good and in poor physical condition. Other potential uses for this new instrument will be discussed.

### 105. The Contribution of Detectable Viruses to the Croup Syndrome

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Of 47 children with infectious croup studied during a one-year period, a virus diagnosis could be assigned in 64%. Type 2 hemadsorption virus infection was found in 36% of all croup patients and was associated with illness throughout the year. Asian influenza virus alone was associated with 36% of croup patients studied during the Asian influenza epidemic and 19% through the year. Nine per cent of croup illness was associated with adenovirus, Type 1 hemadsorption virus, or CA virus.

Type 2 hemadsorption virus infection was found predominantly in boys, but its clinical features were not distinctive.

* By invitation.

### 106. Benign Breast Enlargement in Early Childhood

E. V. PERRIN* and D. H. ANDERSEN, Babies Hospital, New York

Six unilateral breast masses are reported in children from ages 11 months to 8 years. These occurred without accompanying sexual precocity, epiphyseal closure, adrenal tumor, central nervous system abnormality, or known ingestion of hormones by the mother or child.

Three were in males; and three, in females. The swellings increased slowly in size or not at all while observed, and their maximum measurements ranged from 1.3×0.8 to 8×7 cm. Bone age films and urinary 17-ketosteroids in two cases were normal, and a buccal mucosal smear of one child was consistent with its external sex.

In one case only was a definite history of breast enlargement since birth obtained, and this was in the 11-month-old child.

The masses were freely movable, irregularly nodular in two cases, rubbery, and, at operation, gray-pink, glistening, and not obviously encapsulated. One mass was lobular and another contained a single large cyst. Four of the masses were removed in toto or nearly so and two were biopsied.

Microscopically, all of the tumors were composed of dilated ducts lined by multilayered proliferating cuboidal or low columnar epithelium, focally papilliform and nowhere significantly anaplastic.

About the ducts was a myxoid pale concentric fibrous stroma similar to that seen in gynecomastia and in some cases of fibroadenoma in the adult female.

Breast engorgement in the neonatal or prepubertal periods is a well-known phenomenon and is recognized as benign and of endocrine origin. Prolongation of neonatal "mastosis" to 12-14 months has been attributed to initiation of temporary endocrine function in the neonate following the wane of hormones of maternal origin.

In the interim between the two eras of life, such hypertrophy is rare and may be erroneously attributed to malignant neoplasia. Follow-up observations on four of our cases over a period of three to nine years have shown regression or

no change in the masses and no evidence of malignancy.

Mastectomy is unnecessary though occasionally advisable in boys, for cosmetic reasons.

### 107. Dynamics of Fetal Thyroid Chemical Growth and Function in the Rhesus Monkey

DONALD E. PICKERING and
NICHOLIS E. KONTAXIS,* University of Oregon

Festuses, the products of healthy, mature rhesus monkeys, were delivered by cesarean section, killed, and investigated by the parameters to be described at the gestational ages of 75, 90, 105, 120, and 150 days, respectively. $I^{131}$ had been administered intravenously to the mothers at either 24 or 96 hours prior to section. At killing the sera (maternal and fetal), amniotic fluid, and fetal thyroid glands were analyzed for total iodide, thyroxine iodide, mono- and diiodotyrosine iodide, and the specific activity of these fractions where pertinent. Methods employed consisted of modifications of our butanol-extractable iodine (BEI) method in conjunction with chromotography and radiochemistry.

Serum BEI values among mothers and fetuses, irrespective of the duration of gestation, averaged 5.22μg. and 3.34μg. %, respectively. These significant differences were considered to represent differences in thyroxine-binding protein content of their respective sera and should not be interpreted to indicate a relative hormone deficiency among fetuses.

The profound physical growth of the gland and its correlations with body growth are demonstrated in summary form by the following data:

| Gestational Age, (Days) | Animals, No. | Average Body Weight, (Gm.) | Average Thyroid Gland Weight, (Mg.) | Gland Weight (Mg.)-Body Weight (Gm.) Ratio |
|---|---|---|---|---|
| 75 | 2 | 36.6 | 10.9 | 0.298 |
| 90 | 4 | 94.1 | 22.1 | 0.235 |
| 105 | 5 | 149.6 | 42.1 | 0.281 |
| 120 | 4 | 227.8 | 65.4 | 0.287 |
| 150 | 4 | 411.7 | 108.7 | 0.264 |

Over-all chemical growth paralleled physical growth of the gland during the period represented. For example, the total iodide content of thyroids increased from 2μg. at 75 days to 200μg. per gland at 150 days of gestation.

An active hormone synthesis paralleled the over-all growth of the gland throughout the period of study as demonstrated by the fact that thyroxine iodide remained 8.06 ±2.1% of the total iodide in the gland, irrespective of age.

Data relating to thyroxine requirement per unit of body mass per day in the infant monkey suggest that the fetal thyroid gland is primarily responsible for synthesizing hormone only in association with its chemical growth. Studies relating to the $I^{131}$ uptake by the fetal gland and the specific activity and turnover of the thyroxine fraction and that of its precursors mono- and diiodotyrosine corroborate this impression. These observations take on significance relative to the thyroid status of the congenital cretin in utero.

### 108. Studies on the Plasmin-Plasminogen System in Newborn Infants

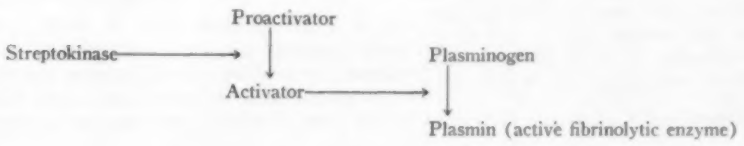
PAUL G. QUIE* and LEWIS W. WANNAMAKER,
University of Minnesota

That the fibrinolytic activity of newborn serum is different from adult serum was first suggested by Boisvert. He found that blood clots from newborn infants showed resistance to lysis in the presence of streptokinase. Since inhibition by placentally transferred antibody was excluded, a deficiency in newborn serum was postulated. Although interest in the fibrinolytic system of the newborn has recently been renewed in other laboratories (Phillips and Skrodelis), the exact nature of the difference in fibrinolytic activity of the blood of newborns has not been defined.

Investigations by Astrup and Müllertz have suggested that the fibrinolytic activation by streptokinase in human blood proceeds by the following simplified scheme at the bottom of this page. It is apparent that the defect in newborn blood may be due to a deficiency in either plasminogen or proactivator.

The present studies confirm the existence of a defect in the plasmin-plasminogen (fibrinolytic) system of the newborn and present evidence that

* By invitation.



the deficiency lies in the level of plasminogen rather than in the level of proactivator. Furthermore, the deficit in plasminogen is more marked in prematures than in full-term newborns.

With use of the casein digestion technique, plasminogen levels were compared in sera from the cord blood of 18 infants and from 14 adults. Cord sera levels were distinctly and consistently lower (average $7.1\times10^{-3}$ Kunitz units per milliliter) than levels in adult sera (average $18.5\times10^{-3}$ units). In cord sera from 11 prematures, the average value was $4.8\times10^{-3}$ units. Euglobulin preparations (i. e., preparations from which inhibitors had been removed) were similarly different in the 18 cord sera (average $14.2\times10^{-3}$ units) and the 14 adult sera (average $30.3\times10^{-3}$ units). In serial determinations on 16 infants, the euglobulin fractions of cord serum and of serum drawn on the first and on the fifth days of life were found to be equal in plasminogen content.

Adequacy of proactivator levels in cord sera was demonstrated in two ways: Excess proactivator provided by adult serum failed to increase the proteolytic activity of six cord sera. Also, 10 different cord sera proved to be adequate sources of proactivator for rabbit euglobulin (known to be deficient in proactivator).

Inhibitor levels as estimated by the difference in proteolytic activity of whole serum and euglobulin tended to parallel the plasminogen levels in both the adult sera and cord sera. Although the resistance of blood clots in the newborn to lysis may be due to an excess of inhibitor(s), no evidence of this was found in estimating inhibitor by this indirect technique.

The present studies have confirmed the existence of a deficiency in the plasmin-plasminogen system of the newborn and present evidence that the deficiency is in the proenzyme and not in the proactivator. The possibility that this enzyme deficiency may play a role in the pathogenesis of diseases peculiar to the newborn period, especially those with fibrin deposition (e. g., hyaline membrane disease), deserves further study.

### 109. Severe Nonhemolytic Jaundice in a Newborn Chinese Infant

KANTI R. RAI,* ARTHUR SAWITSKY,* and PAULA H. ANGELOS,* North Shore Hospital, Manhasset, N. Y.

Introduced by Alfred L. Florman

Severe nonhemolytic jaundice in the newborn period, as described by Crigler and Najjar, in 1952, is a relatively rare condition which was first thought to be only familial and to lead to irreversible brain damage. It is the purpose of this communication to report an isolated instance in a Chinese infant who was found to have convulsions and other signs of kernicterus on the sixth day of life. He was exchange-transfused on the seventh and the eighth days, with subsequent reduction in the bilirubinemia and improvement in the clinical condition.

The patient was a full-term boy of Chinese parentage whose jaundice was first noted at 2 days of age. Between the third and the sixth days he had been home without medical observation. During this time he refused to take feedings and his jaundice increased markedly. When readmitted to the hospital on the sixth day he was deep orange in color, anorexic, lethargic, and in a mild opisthotonos. Soon after admission, he developed generalized convulsions. There was no hepatosplenomegaly. Laboratory findings were consistent with a nonhemolytic process, as evidenced by the absence of spherocytosis, reticulocytosis, or anemia. On admission the total serum bilirubin was 32.4 mg. %, which gradually rose to 40.5 mg. %. The direct fraction was less than 2.9 mg. % at all times. Direct Coomb's test was negative, and the infant's erythrocytes were shown to be completely compatible with maternal serum by the saline, albumin, trypsin, and indirect Coomb's techniques. The mother and the infant were both Type "O" Rh-positive. All the liver-function studies were within normal limits. Exchange transfusions were done on the seventh and eighth days, with resultant improvement in the clinical condition and progressive fall in the blood bilirubin levels. On the 14th day of life the infant was alert, active, and taking feedings very well and had not had any convulsion in a week. The serum bilirubin level on the 14th day was 3.4 mg. %, of which the direct fraction was 0.9 mg. %.

The favorable results achieved by the use of multiple exchange transfusions for the reduction of hyperbilirubinemia, even after the apparent development of kernicterus in an infant on a previous occasion, encouraged us to proceed with the replacement therapy in this patient in spite of the obvious kernicterus present.

This paper emphasizes the role of replacement transfusion in the prevention of kernicterus and infers a longer period of bilirubin exchange between the blood and cerebrospinal tissue than is commonly thought to exist.

### 110. Studies of Cells Cultivated in Vitro from Persons with Genetic Diseases

ARTHUR ROBINSON,* THEODORE PUCK,* and J. H. TJIO,* University of Colorado

Introduced by Henry K. Silver

In previous papers, methods have been described for carrying out the following operations: (*a*) establishment of stable human tissue cultures from skin or other organs, utilizing samples so

* By invitation.

small as to make possible routine culture of somatic cells from any person; (*b*) delineation of the chromosomes of such cells, and (*c*) cultivation of cells for long periods and many generations in vitro without change in chromosome number or constitution (Puck, T. T.; Cieciura, S. J., and Robinson, A.: *J. Exper. Med.* 108:945, 1958; Tjio, J. H., and Puck, T. T.: *J. Exper. Med.* 108:259, 1958). The chromosomes of man, including the X and Y sex-determining members, have been identified and classified on the basis of their morphologies (Tjio, J. H., and Puck, T. T.: *Proc. Nat. Acad. Sc.* 44:1229, 1958). Cell cultures from patients suffering from a variety of genetic diseases have been established and are under study for evidence of chromosomal or biochemical abnormalities. The chromosomal constitution of the somatic cells of normal persons has been found to exhibit remarkably little variation. Delineation of the nuclear sex chromatin by a modification of Barr's technique can readily be performed on such cells grown in vitro and agree with the results of buccal smears taken from the patient directly. Normal chromosomal constitution and sex chromatin have been obtained from cells of all patients so far studied, except for an anatomically female patient with gonadal dysgenesis. This patient possesses only 45 chromosomes with a single X- and no Y-chromosome.

## Discussion

Dr. Lewis Coriell, Camden, N. J.: Dr. Robinson and his group have succeeded in growing many normal cells through 60 or more transfers in tissue culture. The cells grow vigorously and retain their normal morphology and chromosome pattern. These achievements in tissue culture technique have many applications, such as the one just reported. I would like to ask Dr. Robinson to point out the steps in his technique which permit long-term cultivation of normal cells.

This report will stimulate a search for chromosome abnormalities in patients with other genetic defects, such as gargoylism, Marfan's syndrome, and achondroplastic dwarfism, to mention a few. It has already been reported from England and confirmed by Robinson and Puck that one extra chromosome is present in patients with Mongolism. The extent to which genetically controlled characteristics in man can be correlated with an abnormal chromosome number or morphologic abnormality of a chromosome can only be established by careful idiogram studies like those just reported here today.

Dr. Judson J. Van Wyk, Chapel Hill, N. C.: These observations raise a number of questions about the syndrome of gonadal dysgenesis, and we would like more information about the structure of the gonads, genital ducts, external genitalia, and any associated extragenital anomalies which this patient exhibited. We have previously regarded the chromatin-negative individual with gonadal dysgenesis who exhibits a completely female phenotype as the most extreme form of male pseudohermaphrodism. Although in most of these patients the gonad cannot be identified as either a testis or an ovary, we had assumed, in line with the experiments of Jost, that these gonads were actually testes that had failed to develop. Gonadal tissue, when recognizable, often consists only of medullary components. There are a number of individuals who are phenotypically similar to the patients with classical gonadal dysgenesis, except for the presence of slight phallic enlargement, who have recognizable testicular elements in their gonadal remnants, with increasing differentiation of the male genital ducts and external genitalia so that the appearance is that of a cryptorchid, hypospadiac male.

Although this exciting demonstration adequately explains the individual with totally undifferentiated gonads or rudimentary ovaries, it is important to determine whether an unpaired X-chromosome precludes any testicular differentiation whatsoever. If testicular remnants are present, the implications regarding male pseudohermaphrodism are obvious. For this reason it is important to correlate the genotype with the gonadal histology and other features which this child exhibited.

Dr. C. Henry Kempe, Denver: I wonder if it isn't time to cease defining maleness and femaleness in any terms except those in which they were so well described by Drs. Puck and Robinson?

Dr. David Smith, Madison, Wis.: The statement was made that this finding of XO would indicate that these patients represent "incomplete females" rather than genetic males. From the chromosomal standpoint it would seem impossible at present to state whether it is the second X- or the Y-chromosome which is lacking, and therefore these individuals cannot be said to be either female or male in a genetic connotation.

Dr. Lytt I. Gardner, Syracuse, N. Y.: It might be worth while to look at this exciting observation in the context of history. A little over a half-century ago, the relationships of the sex chromosomes to sexual differentiation were elucidated, especially in two animals: the grasshopper (McClung) and the meal worm (Stevens). It has taken about 50 years for human cytogenetics to begin to catch up with the early zoological observations on the lower forms.

At present, basic cytogenetic research by biologists has accomplished a minute mapping of the loci of many mutations on the chromsomes of the fruit fly. Because of the favorable nature of cytological investigation of the larval salivary glands in this insect, it has been possible to define

with great accuracy the locations of mutations on several of this animal's chromosomes. The current advance of new discoveries in biochemical genetics leaves little doubt that soon it may be possible to define the exact nature of the molecular changes at these loci. Thus if our premise is correct that human cytogenetics is following in the footsteps of the discoveries made in lower forms, we may look forward to a bright future in the next decades in human cytogenetics. It does not seem unreasonable to expect that the exact mapping of mutant loci on the human chromosomes will take place in the next 20 or 30 years.

DR. ROBINSON: I agree with Dr. Coriell's comments. I think it's important to remember that the mammalian euploid cell can tolerate minimal deviation from optimal physical and molecular conditions in its environment. It is by attention to minimizing these deviations that the development of aneuploidy is prevented. Particular mention may be made here of the problem presented by the mammalian fetal serum used in preparing the nutrient medium. Batches of this serum vary in their toxicity for the growing cells. When growing large numbers of cells in tissue culture, it may take some time before one realizes that the serum is toxic, and by that time it is too late, because aneuploidy has arisen and overgrown the euploid cells.

For this reason, Dr. Puck has utilized the technique of pretesting each batch of serum by single cell culture before adding it to the nutrient medium used in these experiments. This is a much more sensitive method of picking up promptly the presence of toxic or inhibiting substances in the serum. If there is a plating efficiency of less than 5% or 10% in this pretest, the particular serum tested is *not* added to the nutrient medium.

Great attention must also be paid to maintaining constancy of environment, especially temperature, pH, and relative humidity.

Dr. Van Wyk, you asked about the various phenotypic expressions of this condition (gonadal dysgenesis). This is probably part of a spectrum of many different conditions with varying genotypes.

Our patient did not have webbing of the neck or coarctation of the aorta. She did have dwarfism, sexual infantilism, slight cubitus valgus, and the reverse whorls of the hair on the back of the neck.

Dr. Smith, I think we're getting into semantics. My definition of a human female at present is an individual with the internal and external anatomy normally associated with the female, and with at least one X-chromosome and no Y-chromosomes.

A human male, on the other hand, must possess a Y-chromosome.

This definition of a female is strengthened by the recent experiments of Dr. Russell, in which he succeeded in getting mice with an XO constitution to ovulate.

Dr. Gardner, I can only say that I agree heartily with your comments.

### 111. Studies on the Circulation of Infants with Hyaline Membrane Disease

A. M. RUDOLPH, P. A. M. AULD,*
J. E. DRORBAUGH,* A. J. RUDOLPH,* A. S. NADAS, and C. A. SMITH, Harvard

The possible role of a disturbance in the circulation as a mechanism for the production of hyaline membrane disease was suggested by the findings of Gitlin and Craig. The hyaline material in the lungs of infants dying from the disease was observed to contain protein material derived from the infant's plasma. Increased transudation of plasma into the alveoli could be related to increased pulmonary capillary permeability or to increased pulmonary venous pressure associated with left ventricular heart failure.

As of February, 1959, hemodynamic measurements by means of cardiac catheterization have been made in 40 infants during the first day of life at the Boston Lying-In Hospital. In some of these infants angiocardiographic and dye-dilution studies were also performed. Eighteen of these infants showed no evidence of respiratory distress, and were considered to have normal circulation and respiration; 11 had mild respiratory distress, and 11 had severe symptoms. Eight of the latter group of infants later died and showed autopsy evidence of severe hyaline membrane disease.

Mean pulmonary arterial pressures ranged from 16-50 mm. Hg, but the average pressure in the infants with hyaline membrane disease was considerably lower than in the normal or less severely ill. A significant systemic hypotension was also noted in the severely ill patients.

Observations on pulmonary and systemic blood flow were obtained in 24 infants. In the infants with severe respiratory distress the most striking observation was the presence of a widely patent ductus arteriosus. In the normal infants, and those with mild respiratory distress, either no shunt or only a small shunt through the ductus was demonstrated, although in many cases the catheter was easily passed through the ductus.

The significance of the presence of a large functioning patent ductus arteriosus in hyaline membrane disease will be discussed.

Discussion follows Abstract 161.

### 112. A Study of Heterozygosity in Wilson's Disease

ANDREW SASS-KORTSAK, BENJAMIN S. GLATT,* JOAN M. LEEMING,* and FRANCIS A. MUIR,*
Toronto

Hepatolenticular degeneration is recognized as a hereditary metabolic disease transmitted by an autosomal recessive gene. In many other such conditions the heterozygous carriers show alterations of minor degree and can thus be recognized. The detection of an abnormality in the heterozygote is of obvious value in genetic counseling, moreover it may help to elucidate the basic mechanism of the disease, since the abnormality found in the heterozygote is probably a primary change closely related to the genetic defect.

The present report deals with findings obtained in five families, including 10 parents and 9 sibs of 6 children suffering from Wilson's disease (two patients were brothers). None of the 19 family members had clinical signs or symptoms of central nervous system or liver involvement, and slit-lamp examination did not reveal the presence of Kayser-Fleischer rings. Detailed liver-function studies showed no significant abnormality.

Urinary amino acid excretion was within the range of normal in the six parents and eight sibs so tested. The amount of copper measured in 24-hour urine samples was not increased in the members of these families. A significant decrease in serum ceruloplasmin and total copper content was found in only 3 of the 10 parents and 1 of the 9 sibs. While this may be an expression of the carrier state and has also been reported by others in a few members of such families, the incidence of this abnormality is much lower than the expected carrier rate. (All parents and two-thirds of the sibs are expected to be carriers.)

To detect more subtle alterations in copper metabolism, the response to oral administration of a single dose of $Cu^{64}$ was studied in four members of one of these families. A comparison of these results with those obtained by us in the two cases of Wilson's disease and with the findings of Stanley, Morell, and Scheinberg in 16 normal persons revealed the following findings: 1. All four members of the family showed evidence of incorporation of $Cu^{64}$ into ceruloplasmin and thereby behaved differently from cases of Wilson's disease who failed to do so. 2. In three of the four members of the family (both parents and one sib) the rate of incorporation of $Cu^{64}$ into ceruloplasmin was slower than in the normals. The incidence of the latter abnormality, in one family at least, is in keeping with the expected carrier rate, and it is noteworthy that both parents showed this abnormality, whereas only one of each three pairs of parents had reduced serum ceruloplasmin levels in our series of five families. Moreover, of the one pair of parents who both showed a decrease in the rate of incorporation of copper into ceruloplasmin only the mother had a low serum ceruloplasmin level, while the father's level was normal.

In addition to our earlier observation of normal serum ceruloplasmin levels in a proven case of Wilson's disease who nevertheless showed no incorporation of copper into ceruloplasmin, the above findings would suggest the following conclusions: 1. The rate of incorporation of copper into ceruloplasmin may not be solely dependent on the rate of synthesis of this protein. 2. A decreased rate of incorporation of copper into ceruloplasmin is more closely related to the basic defect in Wilson's disease than the change in the rate of synthesis of ceruloplasmin, as can be judged from the blood levels of this protein.

* By invitation.

### 113. Bilirubin Metabolism in the Fetus

R. SCHMID,* S. BUCKINGHAM,* L. HAMMAKER,* and G. MEDENILLA,*
Harvard and Boston University
Introduced by Sydney S. Gellis

The manner in which bilirubin is handled in the fetus has not been clarified. In fetal liver, the enzymatic mechanism for glucuronide formation is poorly developed, the defect extending into the first few days of postnatal life. Hyperbilirubinemia of the newborn is thought to be related to the "immaturity" of this system. That the placenta or the maternal liver may handle fetal bilirubin is suggested by the observation that even in erythroblastosis a significant rise in serum bilirubin concentration usually occurs only after the cord is clamped.

Studies were carried out to determine whether fetal bilirubin is conjugated in the placenta or in the maternal liver.

Attempts were made to demonstrate glucuronide formation in human (term and cesarean section) and rat placenta. However, tissue slices, homogenates, and microsomal preparations incubated with bilirubin, *o*-aminophenol or *p*-nitrophenol in the presence of UDPGA failed to form glucuronides. Likewise, human placenta perfused supravitally did not conjugate bilirubin.

Next, the fetal-maternal transfer of bilirubin in guinea pigs near term was studied, with use of a modification of Dancis' method (*J. Clin. Invest.* 37:1373, 1958). A maternal vein was cannulated near its placenta to permit continuous sampling. The corresponding fetus was delivered into a saline bath; an umbilical artery was cannulated, and the fetus was isolated. Bilirubin dissolved in albumin or guinea pig plasma together with iodinated albumin or chromated guinea pig erythrocytes were

injected into the fetal placental circulation. Maternal blood samples were taken at intervals for estimation and chromatography of bilirubin and for determination of radioactivity.

Unconjugated bilirubin and iodinated albumin were rapidly transferred, reaching peak concentrations in maternal blood two to four minutes after injection. Bilirubin injected into a maternal vein (jugular) rapidly appeared in fetal blood. Conversely, if chromated red cells and bilirubin were injected on the fetal side, only bilirubin appeared in the maternal vein. When conjugated bilirubin (human bile) was injected, the rate of transfer was much smaller.

These experiments indicate that unconjugated bilirubin can be rapidly transferred from the fetal to the maternal circulation.

In view of these findings, the possibility was considered that the inadequate development of the fetal conjugating system could be due to low substrate concentration. Large amounts of anthranilic acid (a compound excreted chiefly as an acyl-glucuronide) were repeatedly injected into rats during the last quarter of gestation. Substantial substrate concentrations were found in fetal livers even 12 hours after administration. The animals given injections and a comparable number of saline controls were decapitated shortly before term, and microsomes were prepared from fetal and maternal livers. These were incubated with *o*-aminophenol and UDPGA, and the amount of *o*-aminophenyl glucuronide formed was estimated. No difference in enzymatic activity was found between fetal microsomes of mothers treated with saline or with anthranilate. Glucuronide formation was approximately four times less in fetal than in maternal livers. These findings suggest that the fetal glucuronide-forming system cannot be induced by the presence of substrate and that the "immaturity" of the liver must have another explanation.

## Discussion

Dr. Ira M. Rosenthal, Chicago: Dr. Schmid asked me to add a recent observation of his colleague Dr. Kurt Isselbacher. Dr. Isselbacher observed a woman with biliary cirrhosis who during her pregnancy had hyperbilirubinemia with both conjugated and unconjugated bilirubin. At delivery a sample of cord blood contained only unconjugated bilirubin.

Dr. William J. Waters, Syracuse, N. Y.: The critical question appears to me to be how much of a factor placental clearance of bilirubin is in the clinical state. Therefore, I would like to ask if there is any information on the ability of the fetus to produce bilirubin in utero. From some clinical observations we have made it is suggested that, although hemolysis may take place in utero with some bilirubin production, the major portion of the latter is produced postnatally. Thus, more knowledge is needed concerning the pathways from hemoglobin to bilirubin, particularly in utero. Faulty metabolism in the latter situation might explain why the newborn infant is not particularly jaundiced at birth rather than placental clearance of this pigment as has been suggested in the past.

Recently we observed a mother at term with liver disease whose serum bilirubin was 10.6 mg. %, of which 8.4 mg. % was conjugated (direct) and 2.2 mg. % unconjugated (indirect). The umbilical vein revealed a total of 2.3 mg. %, of which 0.75 mg. % was conjugated and 1.5 mg. % unconjugated. After birth the infant's bilirubin course was followed and rose to a high of 12.7 mg. % at five days of age of which 11.4 mg. % was unconjugated. Subsequent to this peak, the level gradually decreased. Therefore, in this instance conjugated bilirubin did not seem to be passing from the mother's circulation through the placenta into the infant's circulation.

Finally we have studied the stools of an Rh-negative mother with a bad clinical history. A four-day collection was obtained two weeks prior to delivery, and the total urobilinogen values were low normal. This further suggests that placental clearance of bilirubin may not be a significant factor in the lack of hyperbilirubinemia at birth; rather, it may be the failure of production in utero.

Dr. Richard L. Day, New York: I was waiting for Dr. Johnson. Since she doesn't seem to be rising, I will ask the question that she ought to be asking.

In the Gunn rats, the fetus has a level of bilirubin which is very much less than that of the mother. The mother may have four to six times as high a level of indirect bilirubin in the serum as the fetus. If the fetus cannot excrete bilirubin, and if it passes through the placenta, I don't see why the level wouldn't be the same on both sides of the placenta, or at least higher than it is.

Dr. Robert E. Cooke, Baltimore: In view of Odell's studies on diffusible bilirubin, would you comment as to whether or not you could affect movement of bilirubin with changes in protein concentration, for example, in the mother or in the fetus? Also, what effects on bilirubin movement were observed with the use of protein-binding inhibitors, such as sulfonamide. In answer to Dr. Day, I would think we might ask him whether or not the protein concentrations are very different in the maternal and fetal sides of the Gunn strain of rats.

Dr. Richard L. Day, New York: Dr. Johnson mentioned last night that the protein levels of the rat fetus are low, but I understood that Dr.

Schmid's group, Dr. Buckingham, injected bilirubin which was already attached to albumin, is that not so? In the fetal Gunn rats, where is the bilirubin? It's not in the tissues, and not in the blood.

DR. LOIS JOHNSON, New York: We performed a cesarean section on a jaundiced mother rat mated to a jaundiced male at about 18 days' gestation. In such a mating all of the fetuses must be genetically deficient in glucuronyl transferase. The serum bilirubin of the mother rat at the time of section was 13.8 mg/100 ml. Her total serum protein was 7.5 gm/100 ml. The fetuses weighed approximately 3.5 gm. each. There was very little variation from fetus to fetus in serum bilirubin and total protein levels. The average value for serum bilirubin was 1.8 mg/100 ml.; that for total protein was 2 gm/100 ml.

The usual period of gestation in the rat is 21 days. The young are born in a relatively immature state. The life span of the adult rat red blood cell is 80 days as compared to a gestation period of 21 days. It would seem reasonable to assume that relatively little bilirubin is formed before birth in the rat and that the serum bilirubin level of the fetus is largely a reflection of the mother's bilirubin level, modified perhaps by the levels of serum albumin in the mother and fetus. Such a concept is supported by our observation that homozygous deficient rat fetuses born to nonjaundiced females mated to jaundiced males, cannot be clearly distinguished from the heterozygote fetuses on the basis of serum bilirubin levels. Furthermore, the serum bilirubin levels of normal rat fetuses delivered of normal females mated to normal males, can be elevated to approximately 1.5 mg/100 ml. by the administration of indirect bilirubin to the mother for the hour preceding cesarean section. (Normal rat serum bilirubin: 0.2 to 0.5 mg/100 ml).

The human baby is in many respects more mature than the rat baby at birth, for example, in respect to serum protein levels. The gestation period is much longer than the life span of even the normal adult red blood cell. Presumably the load of bilirubin to be disposed of during fetal life is proportionately greater, even without such complications as hemolytic disease. In normal human newborns the amount of bilirubin in the first meconium has been found to be inversely proportional to the cord bilirubin and the severity of neonatal jaundice. Term infants suffering from erythroblastosis and/or liver disease have been reported with cord levels of both direct and indirect bilirubin exceeding that of the mother, serum protein levels in mother and offspring being about the same. It would appear that in human babies near term, in contrast to rats near term, the fetal liver is the more important factor in determining the serum bilirubin level. The situation early in gestation is probably quite different.

DR. BUCKINGHAM: First, a comment on the amount of bilirubin formed in the fetus. This question can obviously not be answered, since neither the erythrocyte life span is known during the intrauterine period, nor can it be determined whether the hemoglobin is broken down to bilirubin.

We are aware of Dr. Johnson's observations on newborn Gunn rats, but we are unable to explain it. I wonder whether Dr. Johnson or Dr. Day has an explanation?

With regard to the question whether sulfonamides have been used, the answer is no. The experiment was designed to study the permeability of the guinea pig placenta for bilirubin, but we did not study the rate of transfer or the relative pigment distribution in the fetal and maternal circulation.

### 114. A Factor in Normal Human Plasma Which Stimulates Platelet Production: Chronic Thrombocytopenic Purpura Due to Its Deficiency

IRVING SCHULMAN, MILA PIERCE,* ABBY LUKENS,* ZINET CURRIMBHOY,* and ELEANOR FORT,*
Northwestern and the University of Chicago

The existence of a humoral factor which stimulates erythropoiesis is now well established. The studies to be reported demonstrate the existence of a plasma factor which stimulates platelet production in a case of chronic thrombocytopenic purpura due to its deficiency.

The patient investigated is an 8-year-old girl with a documented history of thrombocytopenic purpura dating to age 2 years. Splenectomy at age 3 years failed to give improvement, as did therapy with corticotropin (ACTH) and corticosteroids. Studies in the postsplenectomy period demonstrated that the administration of fresh or stored blood was invariably followed by an extraordinary rise in platelet count to levels which usually ranged from 500,000 to 900,000 per cubic millimeter. Similar results have been obtained with the use of fresh-frozen plasma in over 25 separate experiments. In the absence of plasma infusions, platelet counts are maintained at levels of 20,000 to 70,000 per cubic millimeter. Of great significance is the fact that plasma from a child with acute idiopathic thrombocytopenic purpura was also effective in producing a rise in platelets. The patterns of platelet rise and fall are remarkably constant with the magnitude of response being proportional to the dose of plasma, peak responses being obtained with approximately 5 ml. per kilogram.

* By invitation.

The patient presented and unusual opportunity to study numerous aspects of thrombopoiesis and platelet function. Serial marrow studies demonstrated that plasma infusion is followed by striking megakaryocyte maturation and platelet production which appears within 48 hours after injection. Analysis of the characteristic curves permitted estimation of platelet life span. Intercurrent infection was found to inhibit megakaryocyte maturation and to increase the rate of platelet destruction. The ability to produce platelet populations at will provided the opportunity to study various platelet functions as related to their age.

Administration of the mother's plasma produced a platelet response identical to those obtained with normal plasma. The father's plasma, in equal amounts, induced a platelet response significantly lower than that obtained with the mother's plasma or with 20 different normals. These results suggest that the defect is genetically determined.

## Discussion

DR. NATHAN J. SMITH, Madison Wis.: For those of us interested in the demonstration of a "thrombocytopoietin" Dr. Schulman's excellent presentation is of greatest interest. The study of this patient again demonstrates that the "experiment of nature" is often the most revealing model for new knowledge, more convincing by far in this case than our futile efforts to demonstrate a "platelet-stimulating factor" in the laboratory.

Of particular interest are the observations concerning idiopathic thrombocytopenic purpura. Recently we have been thinking that there must be some inhibition of platelet production in this disease as well as excessively rapid removal of platelets from the circulation. This would be true, if the work of Craddock et al. showing that they cannot remove platelets rapidly enough to produce persistent thrombocytopenia in the dog is applicable to the human with ITP. Could ITP then be due to a lack of thrombocytopoietin? Evidently the patients with ITP from whom Dr. Schulman received some test plasma had whatever was needed in their plasma to stimulate platelet production.

DR. COCHRANE, Halifax, Nova Scotia: I would like to compliment Dr. Schulman on his very interesting piece of work. I would like to first comment that we have administered plasma taken from patients with "essential thrombocytosis" to one patient 1½ years of age suffering from idiopathic thrombocytopenia for eight months. This patient was not unlike the one described by Dr. Schulman and had failed to respond to prolonged steroid and hormonal therapy. There was an initial increase in the platelet count from 8,000 to 30,000 after the first plasma infusion. The platelet count of the infused plasma was 600,000. Repeat plasma infusions did not produce an increase in the platelet count, and the infant died. I wonder if you could comment on the administration of plasma from cases of "essential thrombocytosis" to cases of idiopathic thrombocytopenia? Secondly, I wondered if any change in the platelet count occurred following simply the administration of such substances as PVP, Dextran, or Introdex?

DR. EDNA SOBEL, New York: It is interesting and curious that the erythropoietic factor of the anterior pituitary gland seems to be effective on oral administration. I am wondering whether there is any possibility that plasma given by mouth would stimulate platelet production in a patient with idiopathic thrombocytopenic purpura. If you should find such an effect, the search for the active substance would be both widened and narrowed. For example, hematopoietin, which is thought to be a glycoprotein (Gordan, A. S.: *Physiol. Rev.* 39: 1-40, 1959), probably would not be effective on oral administration if the protein moiety is essential; if the carbohydrate portion is the active factor, it might be absorbed from the gastrointestinal tract.

DR. A. LEONARD LUHBY, New York: There is no doubt that the astute follow-up of the rare experiment of nature, such as Dr. Schulman and his collegues have done here, often teaches us things which are not possible to learn by man-made experimental designs. This has been particularly true and fruitful in the elucidation of the plasma-coagulation processes.

With regard to the "erythropoietic stimulating factor of the anterior pituitary" mentioned by Dr. Sobel, it bears emphasis that the erythropoietic stimulating factor most research groups as well as our own have been working with has been found only, I believe, in plasma or urine. The "pituitary" factor now appears to be contamination with ACTH. The plasma or urine erythropoietic stimulating factor has not been shown, in the experiments in my laboratory or those of my colleagues, A. S. Gordon, S. J. Piliero, or J. Winkert, to be effective when administered orally. In addition, no one has yet observed an instance of a congenital anemia due to the lack of erythropoietic stimulating factor. The latter is not only ineffective in congenital pure red cell anemia, but most such patients have an increase of erythropoietin, indicating the defect lies elsewhere.

I would like to ask Dr. Schulman if they have considered the possibility of a circulating inhibitor of the normal plasma thrombocyte-stimulating factor which appears to be deficient in their patient. Transfusion of their patient's blood or plasma to a normal recipient may depress the latter's platelet formation if an inhibitor is present.

Also, I wonder if you can tell us the actual time of onset of hemorrhagic phenomena. Did it begin

within a few days or not until many weeks after birth? I ask this because I would suspect that the mother's plasma thrombocyte-stimulating property, which you indicated was normal, would traverse the placenta and protect the fetus and newborn, if the deficiency were congenital. If not, there is a possibility an inhibitor developed later in life.

You also said that following the splenectomy a number of years ago, the patient's platelet count rose. It was not indicated whether this rise was associated with a transfusion administered before or at the time of the operation, or whether, as occurs in many patients with idiopathic thrombocytopenic purpura, a platelet increase occurred following splenectomy or extensive operative procedures.

Lastly, have you observed development of resistance to transfusion of normal blood or plasma? Because this child was transfused so often and over many years, an "antibody" may have developed to a blood factor for which the patient was markedly deficient.

DR. INGRID GAMSTORP, Boston: Had the father had any symptoms of increased bleeding tendency? Was his platelet count normal? Had the patient siblings, and, if so, how many? Had they symptoms of the same type as the patient.

DR. SCHULMAN: Dr. Aballi raised the important question concerning the lack of relationship between platelet numbers and platelet function. In this regard we made one interesting observation. On one occasion the patient was given plasma when her platelets were 20,000 and when spontaneous hemorrhages in the skin were occurring. As her platelets rose to 100,000, bleeding stopped. However, on the downward limb of the platelet cycle, as the platelets fell to 100,000, bleeding again recurred. This suggested that the young platelets were functionally better than the old platelets even though their numbers were equal.

The observation that fresh blood may improve capillary fragility independent of platelet count is most interesting, but we have no data on this aspect in our patient.

Dr. Nathan Smith mentioned that he was disappointed that the plasma of the child with ITP-induced production of platelets in our patient. We were not disappointed in this result at all and rather expected it. The child whose plasma was tested had typical acute idiopathic thrombocytopenic purpura, in which, as you know, over 75% of children recover spontaneously. In the patient presented today, however, we were dealing with chronic thrombocytopenic purpura of long duration and refractory to splenectomy. We anticipated that the child with acute ITP would have at least normal amounts of platelet-stimulating factor and perhaps even greater than normal amounts. Whether other children who demonstrate chronic ITP prove to have the same type of defect as our patient remains to be seen in further studies.

Dr. Cochrane brought up the question concerning the effect of plasma from individuals with thrombocytosis. This is certainly one study which we are very anxious to do on our patient. It would seem to me at this time, however, that the effect of such plasma would depend largely upon whether or not the patient had a deficiency of platelet-stimulating factor as the basis for his thrombocytopenia. If the cause of the thrombocytopenic purpura is not due to a platelet-stimulating factor, I would not anticipate that plasma from an individual with thrombocytosis would have any effect. In our patient, however, in whom deficiency of a platelet-stimulating factor seems to underlie the thrombocytopenia, we anticipate that plasma from a patient with thrombocytosis would produce a greater response than an equal amount of normal plasma. In answer to the second question, we have not tested any other plasma expanders.

Dr. Luhby mentioned the fact that there has not, as yet, been a patient described in whom there has been lack of erythropoietin as a cause of anemia. When the erythropoietic factor was first described, it was anticipated that at least some cases of congenital anemia would be due to deficiency of this factor. As Dr. Luhby points out, however, none has been found as yet. There has been, heretofore, only suggestive evidence that a thrombocyte-stimulating factor existed. In our studies to date we feel that the existence of such a factor has been demonstrated and that our patient represents chronic thrombocytopenia due to its deficiency. In regard to the question concerning the possibility of an inhibitor, we feel that the quantitative relationship which has been demonstrated between the dosage of plasma and a subsequent platelet rise speaks against the presence of an inhibitor.

The history in this child indicates that hemorrhagic manifestations were present at the moment of birth. Therefore, there does not seem to be any protective effect from the mother's plasma.

The question concerning the relationship of the spleen and of splenectomy to the platelet response in this patient is most interesting. When we went back to the presplenectomy data in this child there was an indication that in the preoperative period a slight but definite rise in platelet count followed administration of blood transfusions. This was observed on two occasions. However, in the presplenectomy period the maximum rise in platelets following a transfusion was to a level of 100,000 per cubic millimeter. In the postsplenectomy years equal amounts of blood or plasma have produced platelet rises to over 600,000 per cubic millimeter. This suggests to us that the platelet-stimulating factor produces a highly magnified effect in the

absence of the spleen. We are now involved in studying this aspect further.

At the present time, the child has received plasma at approximately monthly intervals for the past five years. Thus far we have not noted the development of any indications of resistance.

Dr. Sobel's comment concerning attempts to administer plasma orally is most interesting, and we certainly plan to do this. The apparent stability of the platelet-stimulating factor makes us feel that it will be possible to concentrate and store the appropriate fraction for extensive trial.

The father's platelet count has been done repeatedly and ranges from 180,000 to 227,000 per cubic millimeter, which, in our laboratory, is at the lower limits of normal for an adult. He has not had any evidence of hemorrhagic disorder.

### 115. Generalized Lipoatrophy, Cirrhosis, Accelerated Growth, and Insulin-Resistant Hyperglycemia: Longitudinal Observations and Metabolic Studies

ROBERT SCHWARTZ, IRWIN A. SCHAFER,* and ALBERT E. RENOLD,* Harvard and Baker Research Laboratory, Boston

Detailed clinical observations have been made on a 14-year-old girl who first presented with complete absence of subcutaneous fat tissue at 4 months of age. Hepatomegaly and cirrhosis proven by biopsy were apparent by 2 years of age. An accelerated growth rate, evidenced by increased height and advanced bone age, was recorded from infancy until the onset of hyperglycemia, at 12 years of age.

The disordered carbohydrate metabolism in this patient was characterized by (1) marked resistance to ketoacidosis, (2) insulin resistance as evidenced by high insulin tolerance tests (50 units or 1 unit per kilogram of intravenous crystalline insulin produced only a slow fall in blood glucose levels), (3) adequate control of hyperglycemia only when caloric intake was restricted in the presence of the usual daily 200 unit dose of insulin.

In addition repeated assays for plasma and/or serum insulin-like activity by the rat epididymal fat-pad technique have been high normal or markedly elevated for as long as five months after the withdrawal of exogenous insulin. $I^{131}$ insulin tolerance test with use of nine units of carrier insulin resulted in 87% of plasma insulin being protein bound 24 hours after intravenous administration. The degradation curve of the administered $I^{131}$ insulin was extremely slow. Starch electrophoresis performed on a plasma specimen after the administration of $I^{131}$ insulin showed two major peaks of activity in the $\alpha_1$- and fast $\gamma$-ranges. The persistent elevation of plasma insulin-like activity by the rat adipose tissue technique suggests that the insulin "antibody" complex retains biological activity in vitro, at least with respect to this test tissue.

Studies on fat metabolism have shown no hyperlipemia despite persistent hyperglycemia. Repeated determinations of fasting plasma unesterified fatty acid concentrations have been normal. This fraction showed a definite but slow response to intravenous insulin, although the major source of production, namely, subcutaneous fat tissue, was absent. After an oral fat load of 3 gm. per kilogram, no gross defect in the absorption of fat or in the transfer of neutral fat from the plasma to the tissues could be demonstrated.

Studies with 2-$C^{14}$ acetate performed in collaboration with Shreeves and Hennes at Brookhaven, Long Island, revealed a rapid high peak of incorporation of $C^{14}$ from acetate into both cholesterol and triglyceride. However, the disappearance of radioactivity from these lipid fractions was significantly diminished.

Endocrine evaluation of adrenal, thyroidal, and gonadal function have not been remarkable. There has been no hypermetabolism. Review of nine similar cases indicates that accelerated growth is an important feature of this syndrome in children. Adult cases have none of the clinical features of excessive pituitary activity.

Two hypotheses may explain the syndrome: (*a*) a primary hyperfunction of the pituitary with excessive growth hormone production, depletion of fat tissue and secondary hepatic dysfunction; excessive growth could occur early and diabetes later similarly to the experimental diabetes of Young; or (*b*) a primary defect in the mechanisms for the subcutaneous storage of excess calories as subcutaneous fat producing initially increased growth in the presence of normal amounts of growth hormone and followed by disordered carbohydrate metabolism and increased hepatic storage of fat with resultant cirrhosis. In the present case, an absence of functionally normal subcutaneous tissue seems the more reasonable factor.

### 116. Anemia, Hypoproteinemia and Hypocupremia in Pancreatic Insufficiency

NASROLLAH T. SHAHIDI,* FERNANDO MONCKEBERG,* HARRY SHWACHMAN, and LOUIS K. DIAMOND, Harvard

Inadequate hemoglobin synthesis in the presence of hypoproteinemia has been investigated by others in different animal species, such as dogs, swine, and rats. The absence, or very low levels, of protein in the diet of these animals despite adequate iron leads to hypoproteinemia and normochromic normocytic anemia of moderate degree. In addition, the hypoproteinemia could involve the specific metal-binding globulins and result conse-

* By invitation.

quently in hypoferremia and hypocupremia. Recent reports reveal that similar findings can develop in infants as the result either of inadequate diet or an increased rate of protein degradation. The present studies show that such a syndrome can also be one of the early manifestations of pancreatic insufficiency presumably due to inadequate protein digestion. A series of investigations were carried out on two white boys, aged, respectively, 2 months and 2½ months, who were admitted to the Children's Medical Center because of marked generalized edema and pallor. The total serum protein on each patient was markedly reduced (3 and 4 gm. %, respectively), with involvement of albumin as well as globulin levels. Further laboratory procedures revealed absence of trypsin in the stool as well as in duodenal fluid. High levels of electrolytes in sweat confirmed the diagnosis of cystic fibrosis of the pancreas. The anemia present in both children was normochromic, normocytic, and of moderate degree. A bone marrow aspiration revealed normal myeloid elements and megakaryocytes with relative depression of erythroid series. In addition to hypocupremia and hypoferremia the examination of sera revealed a complete absence of iron-binding capacity in one patient and markedly reduced capacity in the other. Immunochemical studies with rabbit antihuman $\beta_1$-globulin serum showed that the iron-binding globulin, although extremely low, was not completely absent. An albumin turnover study with use of albumin labeled with $I^{131}$ showed a normal rate of protein degradation. The administration of pancreatic extract and an increased protein intake, without addition of iron or copper in each patient, resulted in a prompt and sustained rise of reticulocytes. Serum iron and iron-binding capacity as well as the serum copper returned to normal values within a month's period. With the continuation of therapy, the hemoglobin and the total serum protein rose steadily until they reached normal values.

Here then is a new cause previously not reported for anemia and hypocupremia due to hypoproteinemia resulting from cystic fibrosis of the pancreas.

### 117. Enzyme Deficiency in Erythrocytes in Congenital Nonspherocytic Hemolytic Anemia

NASROLLAH T. SHAHIDI * and LOUIS K. DIAMOND, Harvard

Although there are several reports of congenital nonspherocytic hemolytic amenia in the literature, little is known about its pathogenesis. The shortened survival of red blood cells from such patients has suggested an intrinsic defect, the nature of which has remained unknown. Recently a 7-year-old boy and his 5-year-old brother with an unusual hemolytic anemia were studied. Both children had exhibited unexplained hyperbilirubinemia shortly after birth, which cleared up by 10 days in one patient and 3 weeks in the other. Splenomegaly, reticulocytosis, anemia, and erythroid hyperplasia in the bone marrow were present in both patients. This chronic hemolytic process was complicated by acute exacerbation manifested by marked anemia and hemoglobinuria in both children, without any history of known drug ingestion. Suitable tests ruled out congenital spherocytosis, hemoglobinopathy, Mediterranean anemia, or acquired autoimmune hemolytic disease.

The measurement of the reduced glutathione content of the erythrocytes and of its stability upon incubation with acetylphenylhydrazine in vitro revealed markedly abnormal values in both children. Assay of erythrocyte glucose-6-phosphate dehydrogenase likewise showed markedly low values. The erythrocytes from the mother showed a similar metabolic defect but to a milder degree, while those from the father revealed no demonstrable abnormality. Assay of glucose-6-phosphate dehydrogenase was also carried out with erythrocytes from the mother's parents and relatives, but no enzyme deficiency could be detected.

These findings indicate that these children and their mother have a metabolic defect in their erythrocytes similar to that encountered in patients with drug-induced hemolytic anemia and favism. These children demonstrate that glucose-6-phosphate dehydrogenase deficient erythrocytes do not necessarily require the injurious effect of a drug to undergo hemolysis if, as in these cases, the metabolic defect is extremely severe. The exact mechanism whereby these metabolic defects produce hemolysis is unknown. Whether or not they are the only factors remains to be investigated.

The onset of jaundice and anemia shortly after birth in our patients indicates that the injection of vitamin K or the ingestion of naphthalene by the mother in late pregnancy, as reported by Zinkham, are not essential for the production of hemolytic anemia in the newborn infant with glucose-6-phosphate dehydrogenase-deficient erythrocytes.

### 118. Tubular Reabsorption in Sweat Glands in Cystic Fibrosis

MAARTEN S. SIBINGA * and GIULIO J. BARBERO, Children's Hospital, Philadelphia

The concentration of Na in sweat is, as a rule, considerably lower than the plasma Na concentration in the normal person. This difference is much less or nonexistent in cystic fibrosis. Values of Na concentration exceeding those of plasma have been reported in cystic fibrosis. These high values probably are partially due to the method of collec-

* By invitation.

tion but may be real. On anatomical and physiological grounds two functional subdivisions of the process of sweat secretion can be accepted: precursor formation in the "coil" and reabsorption of solutes in the tubule. A means of investigation of the abnormality in cystic fibrosis sweat is offered by the finding that the rate of Na excretion in normal sweat approaches a linear function of the rate of flow (or, of the rate of secretion of a precursor solution) asymptotically with increasing sweating rates. The intercept of this asymptote can be used in estimating tubular reabsorption and consequently provides a way to study the precursor. Without studying the relationship of flow and electrolyte concentration in sweat of patients with cystic fibrosis, conclusions about tubular function cannot be made and information as to the possible site of origin of the high Na concentration cannot be gained. The technique of I. L. Schwartz was utilized for quantitative estimations of localized sweat flow and electrolyte concentration after intradermal injection of methacholine chloride. This method allows correlation between the electrolyte concentration and flow rate.

It was found that good reabsorptive capacity of the tubular part of the sweat glands of patients with cystic fibrosis is present for Na, falling in the same order of magnitude as the normal. The slope of the linear function can be interpreted as the maximal concentration of Na on intense stimulation. The variance of this slope from experiment to experiment and between different patients with cystic fibrosis was studied. It was found to vary considerably with different intakes of NaCl. Values for the slope exceeding plasma Na concentration were repeatedly observed. This slope represents a maximal rather than an arbitrary intermediate performance that may cause the variation frequently found between different sweat samples collected by the conventional methods of sweat collection where the rate of secretion is not used for interpretation.

Excretion rates of Cl, which seem to be of considerable theoretical interest in view of the recently presented electrophysiological evidence (Lundberg) for the "leading role" of the Cl ion in the parotid gland, are being studied.

### 119. Cytology of Human Urinary Sediment as a Diagnostic Aid in the Determination of Estrogen Effect in Children

HENRY K. SILVER, University of Colorado

Although it has been known that the epithelial cells from the distal urethra that are normally present in voided specimens respond to the influence of estrogens in much the same way as does the vaginal mucosa, the study of the cytology of human urine has not been widely used in pediatrics.

The method consists of collecting and centrifuging a voided specimen of urine (preferably the first morning specimen, since this contains more cells), fixing the sediment, and staining it with a single differential stain (S3). With experience, changes in cell structure and staining characteristics may be interpreted with ease.

We have noted that no estrogen effect is present in cells obtained from the urinary sediment of preadolescent children, while cells obtained from pubertal males and females show an estrogen effect which becomes most striking in the postmenarchal girl. The urinary cells of adolescent males tend to show a more striking estrogen effect than is found in older men. As would be expected, estrogen effect in both the full-term and the premature infant is easily demonstrated at birth and persists for several weeks. Cystologic examination has also been of value in studying various disturbances with possible altered estrogen effect, including precocious sexual development, gynecomastia, and hepatic disease.

Examination of the cytologic content of the voided sediment and of the vaginal mucosa obtained by the Papanicolaou method, taken simultaneously, demonstrated a striking similarity with the same cell types found in both specimens and with cells from both areas showing similar morphologic and staining changes in relation to the presence or absence of estrogens. One of the great advantages of examining the urinary smear of the young girl is that it gives information regarding estrogen stimulation without the need for vaginal manipulation and the consequent hazard of undesirable psychic trauma. In boys it offers a simple method of obtaining cells from the anterior urethra, an area which is sensitive to the effect of estrogen or estrogen-like substances. In both sexes, observations on single or serial specimens has diagnostic value in studying various endocrine and nonendocrine disturbances that are accompanied by altered estrogen effect.

The technique of urinary sediment examination will be described in detail, and representative smears of the cells obtained from normal children ranging in age from birth through adolescence as well as those with various abnormalities will be demonstrated and discussed.

### 120. Changes in the Blood Volume of Infants in the First Hours of Life

THOMAS R. C. SISSON, University of Rochester

It has been assumed that a shift of plasma from intra- to extravascular space occurs because the hematocrit tends to increase in the hours following birth. However, the observation that high-plasma-volume levels are present in newborn infants has seemed inconsistent with this assumption.

In order to chart the actual volumetric changes and their direction, 12 normal full-term infants were studied within one hour and again at three, four, or five hours after spontaneous, nonanesthetic delivery. The umbilical cords of the infants were ligated within 30 seconds of the delivery of the buttocks. Plasma-volume determinations were made with use of Evans blue dye (T-1824). Hemoglobin concentration and hematocrit determinations were also done at the same time and from the same samples of venous blood. The total blood and erythrocyte volumes were calculated from plasma volume and hematocrit, using appropriate factors for trapped plasma and body-venous hematocrit ratio.

Total blood, plasma, and erythrocyte volumes increased without exception. At about one hour of age, the average plasma volume was 47 ml. per kilogram, the erythrocyte volume was 40 ml., and the blood volume was 87 ml.

Between 3 and 5 hours of age, however, the plasma volume had increased 17%, to 55 ml. per kilogram; the erythrocyte, volume 21%, to 49 ml., and the blood volume had increased 20%, to 104 ml.

That the hematocrit should have increased in the face of an undoubted expansion of the plasma volume is explained by the fact that the increment of erythrocyte volume was greater than that of the plasma volume.

We do not know from what reservoir the plasma and red cells were drawn. It may be supposed that the extra quantity of whole blood was at first sequestered in the body while hemodynamic adjustments took place. We have assumed that the blood was gradually released to the general circulation upon the enlargement of the pulmonary vascular bed and the establishment of other postnatal circulatory patterns.

The average total blood volume increment of 54 ml., or 17 ml. per kilogram, appeared to be spontaneously added. That such a quantity of whole blood is readily available to the newborn infant would seem to make the artificial transfusion of placental blood not only unnecessary but possibly hazardous. We cannot say what percentage of the total blood mass may be added safely without embarrassment to the cardiovascular and pulmonary circulation.

It was concluded from the results of this investigation that the total circulating blood volume of the newborn infant increases during the hours immediately after birth by an expansion of both the plasma and erythrocyte volumes. If a shift of plasma to extravascular space occurs, it is obscured by the large increase of plasma volume.

### Discussion

DR. IRVING SCHULMAN, Chicago: Dr. Sisson, do you believe that the increase in red cell mass which may be accomplished by stripping the cord may contribute to the development of hyperbilirubinemia?

DR. STANLEY JAMES, New York: We have been following Dr. Sisson's blood volume studies with great interest. It is pertinent that these data should be presented when Dr. Gairdner has suggested just the opposite; that in the majority of infants there is a shift of fluid from the vascular compartment (mainly whole plasma) immediately after birth. However, these conclusions have been based on measurements of hemoglobin and hematocrit rather than the actual blood volume.

An important point which I don't think Dr. Sisson stressed is that the lowest plasma and blood volumes noted have been in babies with respiratory distress. This is not altogether surprising in view of the edema so commonly seen in such infants.

DR. ARTHUR E. MCELFRESH, Philadelphia: The only question that I have for Dr. Sisson is with regard to possible changes in the body hematocrit in contrast to the venous hematocrit during this period of expansion of the lung and quite possibly changes in the volume of other capillary systems throughout the body.

DR. ANDREW SASS-KORTSAK, Toronto: I would like to ask Dr. Sisson whether he determined protein in the samples he removed at one hour or three to five hours.

DR. PHILLIP STURGEON, Los Angeles: Of particular interest to me is the point made by Dr. Sisson relative to the role of the placental transfusion in infant iron nutrition. I concur with him that the contribution of the placental transfusion to an infant's total iron nutrition at one year of age is relatively insignificant. Certainly the pros and cons of the placental transfusion problem should be weighed without regard to infant iron nutrition.

DR. SISSON: Dr. Schulman, I do not know exactly what changes in oxygen tension and saturation have occurred in these infants. With proper equipment increases could possibly be recorded with increase in red cell mass, though this would depend on the cardiac output and rate of arterial flow. The fact that changes in the size of the red cells are fairly rapid after delivery might have some influence also.

I have no data as yet as to the contribution of the increased hemoglobin mass to the concentration of circulating bilirubin. On the average, the hemoglobin mass increased 2.3 gm. per kilogram or 11 gm total. We studied 10 infants who were deliberately made plethoric. All developed a degree of hyperbilirubinemia. However, we found no really good correlation, and I think it would require a larger series than ours to say that plethora would increase the hazard of kernicterus. The likelihood, though, is great.

Dr. James, I do think that the size of the liver may well reflect the amount of blood sequestered before the fourth hour of life. Dr. Usher of Montreal has found that in hypotensive states in the newborn the liver will increase in size markedly. There is reason to suppose that it may accommodate 60 to 70 milliliters. This and a transudation of plasma could account for the lower plasma volumes we have observed in babies with respiratory distress in the newborn period.

Dr. McElfresh, I believe there may well be changes in the ratio of venous to capillary hematocrit in this period of time. The better way of investigating this would be by simultaneous measurement of red cells by isotopic tagging, and plasma with either dye or radioiodinated albumin. We, with our one-sided technique, have not been able to do this very accurately.

Dr. Sass-Kortsak, we have measured the plasma protein of all samples, and have found very little change. It was necessary to do this in order to calculate dye disappearance. We found only minor change in protein at various time intervals, sometimes increased, sometimes decreased.

Dr. Sturgeon, I agree with you that the amount of iron contributed to an infant by placental transfusion is meager. I feel that the changes in physiologic patterns of respiration, hemodynamics, and homeostasis that are caused by such a transfusion are possibly hazardous. This outweighs the slight addition of iron thus gained.

### 121. The Comparative Value of Intramuscular Versus Orally-Administered Iron in the Prevention of the Late Anemia of Prematurity

A. L. Sitarz,* J. A. Wolfe, and
F. H. Von Hofe,* Babies Hospital, New York

Previous observations have shown that premature infants, especially those with lower gestational age, are subject to an early anemia which is resistant to oral iron therapy as well as to an anemia at age 6 to 9 months which responds to oral iron administration.

The purpose of the present study is to demonstrate the efficacy of intramuscular iron in preventing the late phase of the anemia of prematurity.

One hundred infants of birth weight less than 1,800 gm. were studied. Initial blood cell counts were done at the time the subjects reached a weight of 2,100 gm., and alternate infants were then started on either Fer-in-Sol or Imferon (iron-dextran complex). Fer-in-Sol was administered in daily 0.6 cc. doses until the second month, when 0.6 cc. was given twice daily. The dose of Imferon was calculated by using Brown's formula (estimated body weight at one year, the age used, was 10.6 kg.):

$$\frac{\text{12 gm. patient's hgb.}}{100} \times \underset{(8\%\ \text{est. body wt.})}{\text{est. blood vol. (cc.)}} \times 0.0034 \times 1.5 = \text{gm. elem. iron}$$

The following determinations were performed at 2, 6, and 9 months of age: hemoglobin, red cell count, hematocrit, reticulocyte count, and red blood cell indices. The subjects were divided into three groups according to birth weights: less than 1,200 gm., 1,200-1,500 gm., and over 1,500 gm. Groups of untreated infants of comparable weights previously evaluated in the same premature nursery served as controls.

Of the 100 infants started on the study, 20 were lost to follow-up for various reasons. Eighty subjects remained on study at least part of the nine months, and 54 (to date) were on study the full nine months or longer. Approximately an equal number of these patients received Fer-in-Sol as received Imferon.

Hemoglobin values at 6 to 9 months of age showed that parenteral iron was as effective in preventing the late phase of the anemia of prematurity as oral iron.

No untoward reactions of a local or systemic nature were encountered in any of the 50 infants who received Imferon.

It is felt that parenteral iron has a definite advantage in the prophylaxis of iron-deficiency anemia in premature infants when the follow-up may prove difficult because of economic factors, transportation problems, or language barriers.

Parenteral administration of iron before the infant leaves the premature nursery can insure adequate iron stores for the first year of life and obviates the difficulties associated with the daily administration of iron.

### 122. Enzyme Activity in Erythrocytes: Further Comparisons Between Newborns and Adults

Moyra T. Siu * and Ruth T. Gross, Stanford

It has been previously established that certain enzymes (glucose-6-phosphate dehydrogenase, 6-phospho-gluconic dehydrogenase, and aldolase) are high in the erythrocytes of newborn infants and do not fall within the lower adult standards for at least six months (Gross, R. T., and Hurwitz, R. E.: *Pediatrics* 22:453, 1958). It has also been shown in adults that the two dehydrogenases are highest in young erythrocytes and decrease in activity with the in vivo aging of the cells (Marks, P. A., and Johnson, A. B.: *J. Clin. Invest.* 37:1542, 1958). The present study was designed to determine whether the higher enzyme levels in the erythrocytes of young subjects represent enrichment of the blood with young cells or a unique difference in erythrocyte metabolism.

* By invitation.

Two enzymes were evaluated which differ from those previously studied in that they do not show increased activity in the young erythrocytes of adults and do not decrease with the in vivo aging of the cell, namely, lactic acid dehydrogenase and purine nucleoside phosphorylase (Marks). The levels of these two enzymes in the erythrocytes of newborns were compared to those of adults. No significant differences were found in the two groups. Thus in the enzymes which have been studied to date the only ones which have been found to be elevated in young subjects are those which show increased activity in the young cells of adults.

Erythrocytes of newborns and of adults were separated into the oldest and youngest cell populations by the technique of differential osmotic saline fragility. The levels of glucose-6-phosphate dehydrogenase were compared in the young and the old cell fractions of both young and adult subjects. The enzyme was found to be significantly higher in the oldest cells of newborns than in the adults' oldest cells.

These findings suggest that high levels of enzyme activity in the erythrocytes of young subjects are a reflection of a younger mean cell age.

### 123. Proteinuria in Health and Disease

R. J. Slater, B. Brown,* D. Mills,* and P. Azzopardi,* Toronto

Despite the important role of the kidney in the preservation of the plasma proteins, irrefutable evidence substantiating a clear relationship between glomerular filtration and tubular reabsorption of protein by the human kidney has not been presented. Renal handling of proteins as reflected by clearances has been reported by several investigators (Hardwicke, J., and Squire, J. R.: *Clin. Sc.* 14:509, 1955; Chinard et al.: *J. Clin. Invest.* 33:621, 1954; McCrory, W.: Proceedings of the 8th Annual Conference on the Nephrotic Syndrome, Oct., 1956, p. 14). While Hardwicke suggests that tubular reabsorption of approximately 15 gm. per day of protein from glomerular filtrate may occur, Chinard's interpretations suggest a less active tubular role. Our studies on patients with proteinuria have raised certain new possibilities.

The frequent finding of Bence Jones protein as an isolated urinary constituent throws doubt upon any significant tubular reabsorption of a hypothetical 15 gm. per day of protein from the glomerular filtrate. If proteinuria occurs because a tubular reabsorptive maximum is exceeded, then the Bence Jones protein should routinely appear in the mixture with the other plasma proteins of the glomerular filtrate.

Changes in the proportional clearances of proteins of different size have been observed in nephrotic children passing into and out of relapse. Assuming an analogy to a pore filtration system, the quantity of proteinuria as well as the change in clearances may be a function of alterations in the number of pores of different size in the glomerular membrane.

By contrast, in any nephrotic person observed during relapse, unchanging proportional clearances of macromolecules suggest a "fixation" in the characteristics of glomerular permeability. Therapeutic infusion of albumin causes an increase in the plasma albumin and a decrease in plasma globulin levels as well as a marked increase in the urinary protein excretion. Surprisingly, despite decreases in the plasma globulin levels caused by hemodilution, clearances of the globulins increase in direct proportion to the clearance of albumin. The maintenance of identical proportional clearances of specific plasma proteins before and after albumin infusion suggests unchanged pore-size distribution. Such "fixed" characteristics of filtration apply as well to persons with postural proteinuria observed during variations in protein excretion rates.

Since these filtration characteristics are responsible for the nature of the urinary protein mixture, electrophoretic analyses of the urinary proteins made during these functional changes have been of assistance in clarifying clinical problems associated with lymphuria, exercise, and "normal" proteinuria.

It is concluded that in the human the nature of proteinuria is a function of glomerular membrane permeability. Since this concept may be most readily expressed in terms of pores in a semipermeable membrane, quantitative proteinuria may be controlled by total pore permeability, whereas qualitative proteinuria may be determined by the relative population of pores of different sizes. Tubular reabsorption apparently plays a very minor role, implying that in health glomerular permeability to protein is negligible.

#### Discussion

Dr. Walter Heymann, Cleveland: Again I would like to take advantage of this occasion to make a plea for striking the term "albuminuria" from every publication dealing with protein excretion in the urine. By now, and as Dr. Slater again has shown, we very well know that we do not deal with an exclusive or even always predominant excretion of albumin in the urine. When occasionally 60% of all urinary proteins may be globulins, the term "albuminuria" leads to erroneous conceptions and should be replaced by "proteinuria."

I would appreciate a comment from Dr. Slater on how he believes his and our previous work can

* By invitation.

be reconciled. Some three to four years ago we studied, quantitatively, using the Tiselius electrophoresis, the excretion of albumin and globulin in the urines of children with acute glomerulonephritis and with the nephrotic syndrome, during different phases of their disease. We found, similarly to what had been published by Blackman a few decades ago, a urinary excretion of more than 2 gm. of globulin per 24 hours, or an excretion of globulins in excess of 45% of the total urinary protein output as usually seen in progressive nephrotic diseases. If obtained regularly over longer periods of time, this may serve as a "signum mali ominis." The albumin to globulin ratio in the urine did not, furthermore, show any regular alterations under steroid-induced diuresis. How can this be brought in accord with the observations that you made?

DR. ROBERT J. MCKAY, Burlington, Vt.: Dr. Slater, do you think your electrophoretic patterns of urinary proteins might possibly have any diagnostic use?

DR. MALCOLM A. HOLLIDAY, Pittsburgh: One alternative explanation to the one offered is the filtration of larger quantities of protein and back diffusion in some way of protein in the same pattern in which it is filtered. Additional support for the hypothesis that Dr. Slater proposed is provided in the earlier evidence of the filtration of proteins in the nephrotic patients given albumin.

DR. CLARK D. WEST, Cincinnati: As I saw your data on patients with nephrosis, the ratios of the clearance of albumin to that of γ-globulin changed almost immediately when the patient was started on steroid therapy. I wonder whether this might not be an effect of steroid directly on the glomerular membrane. It should be remembered that in the rabbit cortisone can produce pathological changes in the glomerulus and proteinuria. Has Dr. Slater placed a nonnephrotic patient with proteinuria on steroid to see whether a similar change in ratio would occur?

DR. SLATER: Dr. Heymann, it may be difficult to compare your findings of unchanging albumin/globulin ratios of urinary protein with our data on changing clearance ratios of albumin/γ-globulin. Our measurements estimated discrete proteins by immunologic means, whereas you carried out electrophoretic measurement of the total globulins in urine. During progress from relapse to remission in nephrosis, there occurs (1) a decrease in the excretion of urinary protein, (2) an increase in the serum albumin/γ-globulin ratio, and (3) a decrease in the urine albumin/γ-globulin ratio. These changes are magnified when expressed as clearances. The alterations must reflect changes in either glomerular or tubular handling of protein. Considered in association with the findings in other situations, i.e., albumin infusion, we considered it more likely that changes in glomerular permeability might be responsible for our findings.

Dr. McKay, we have not gained appreciable diagnostic assistance from electrophoretic analyses of urinary proteins, with the following exceptions. Bence Jones proteins are not always readily defined by their solubility characteristics. They are resolved readily by electrophoresis. In addition, we are making observations on the nature of asymptomatic persistent proteinuria in contrast to postural proteinuria. Flynn (*Lancet* 2:978 [Nov.] 1958) has published a small series of analyses in which he suggests that the pattern of proteinuria associated with congenital tubular disease may be different from other types of proteinuria.

Dr. Holliday, the results of our studies permit only an indirect estimation of the influence of glomerular filtration versus tubular reabsorption on proteinuria. While back diffusion of protein into the tubule from the glomerular filtrate may occur as you suggest, an alternative hypothesis is an active reabsorptive system with specific enzymes controlling absorption of specific proteins or groups of proteins. Considering the constancy of albumin/γ-globulin clearance ratios noted during extreme variations in protein excretion associated with albumin infusions in nephrotic patients, the possibility of such rapid and marked adaptability of a specific enzyme system controlling reabsorption seems to us less likely than a tubular reabsorptive process that is proportional and nonspecific in nature. I do not believe one can do more than theorize from these studies.

In regard to our feeling that in health there is relatively little glomerular filtration and tubular reabsorption of protein, Dr. Lathem has recently reported (*J. Clin. Invest.* 38:652, 1959) no glomerular filtration of hemoglobin below the haptoglobin saturation level and thereafter minimal, if any, tubular reabsorption of the plasma free hemoglobin filtered by glomeruli.

Dr. West, we have not yet studied the influence of cortisone upon proteinuria in patients other than those with nephrosis.

## 124. Total Anomalous Pulmonary Venous Return: Infradiaphragmatic Type, Clinical Manifestations

BLANCA SMITH,* THOMAS R. FRYE,*
and W. A. NEWTON JR.,*
Children's Hospital and Ohio State
Introduced by Warren E. Wheeler

The present study is an analysis of the clinicopathological features of the two main varieties of anomalous pulmonary venous return: supra- and infradiaphragmatic.

Twenty-two cases of total anomalous pulmonary venous return were autopsied at the Columbus

* By invitation.

Children's Hospital between 1952 and 1958. Of them, 10 were unassociated with other major cardiac malformations.

Emphasis is placed on the infradiaphragmatic type, of which there are only 21 cases reported to date.

This anomaly, with the pulmonary veins draining into the inferior vena cava or portal vein, was encountered in 9 cases of the 22, but in only 5 was it present as an isolated anomaly.

In reviewing the literature one is impressed by the fact that the cases of the isolated infradiaphragmatic variety are almost invariably diagnosed at autopsy. Undoubtedly, the paucity of findings referable to the heart and the inconspicuous heart outline on x-ray are the reasons.

This is in clear contrast with the supradiaphragmatic variety, for which the pathognomonic x-ray appearance of the enlarged heart greatly facilitates the diagnosis. With the aim of defining the common clinical features, which could help to establish diagnosis, the five cases of the infradiaphragmatic variety were reviewed in detail and compared with the previously reported data.

All five were newborns, three boys and two girls, ages ranging at admission from 14 days to 5½ weeks.

The reasons for the hospitalization were severe dyspnea and deep cyanosis that had onset at birth in two of the cases and at the third and fifth weeks in the other three. The heart was not enlarged except in one case, which entered the hospital in advanced heart failure. Heart murmurs were absent in four of the five cases. The interval between the onset of heart failure and death averaged four days, with a maximum of nine days. The age at death was 16 days in the youngest and 5½ weeks in the case with the longest survival.

The chest roentgenogram showed, in all cases, a marked increase of pulmonary vasculature appearing as a fine, diffuse granulated mottling of the lung parenchyma. The liver was moderately enlarged in two of the cases.

At autopsy all of the cases had the pulmonary veins connecting to a vertical common vein that descended to the abdomen, piercing the diaphragm alongside the esophagus and draining into the portal vein, ductus venosum, or inferior vena cava.

Our conclusions were as follows: 1. The supra- and infradiaphragmatic varieties of total anomalous pulmonary venous return are two strikingly different entities in their clinical manifestations. 2. With one exception, the 21 reported cases have been diagnosed at autopsy. 3. There is sufficient evidence accumulated from previous reports by other authors, plus the five cases here analyzed, to substantiate the following diagnostic criteria for suspicion of total anomalous pulmonary venous return, infradiaphragmatic variety: newborn with deep cyanosis and severe dyspnea, showing at x-ray of the chest a normal size heart and a typical diffuse mottling of the lungs.

### 125. Urinary Ketosteroid Sulfates and Glucuronides

J. Darrel Smith,*
Children's Hospital of Philadelphia
Introduced by Walter R. Eberlein

The isolation of sulfate- and glucuronide-conjugated 17-ketosteroids from urine by a method of adsorption chromatography on alumina described by Crepy et al. (*Acta endocrinol.* 24:233, 1957) has permitted an analysis of methods of hydrolysis of urinary ketosteroid conjugates.

The total sulfate- and glucuronide-conjugated 17-ketosteroids obtained by column chromatography were measured by the Zimmerman reaction, with corrections made for the increased color development of the conjugates. Hydrolysis of the 17-ketosteroid sulfates was accomplished by the relatively simple solvolysis procedure described by Burstein and Lieberman (*J. Biol. Chem.* 233:331, 1958). Hydrolysis of the ketosteroid glucuronides was carried out with β-glucuronidase (beef liver) in acetate buffer (pH 4.5). The total urinary 17-ketosteroid values in 10 adults, obtained by hydrolysis of urine with approximately 9.25% (v/v) sulfuric acid at 80 and 100 C for 10 minutes, were compared (see tabulation below).

In most experiments, the ketosteroids liberated by the fractional hydrolytic procedures correlated well with the isolated conjugates. However, the correlation of the total ketosteroids released by acid hydrolysis was best when performed at 100 C.

* By invitation.

| | Conjugates | | Glucuronides, β-Glucuronidase; Sulfates, Solvolysis | | Hydrolyzed | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $H_2SO_4$ (80 C) | | $H_2SO_4$ (100 C) | |
| | Range | Ave. | Range | Ave. | Range | Ave. | Range | Ave. |
| Sulfates | 1.2-11.9 | 7.7 | 3.2-11.5 | 6.7 | | | | |
| Glucuronides | 3.0-15.0 | 10.6 | 3.3-16.0 | 9.7 | | | | |
| Total | | 18.3 | | 16.4 | 5.6-14.7 | 9.7 | 11.5-30.3 | 18.4 |

The chromatographic separation on paper of the individual 17-ketosteroids released by the various hydrolytic procedures will be correlated with these results.

### 126. Free and Conjugated Dehydroepiandrosterone in Human Urine

J. Darrel Smith,*
Children's Hospital of Philadelphia
Introduced by Walter R. Eberlein

Dehydroepiandrosterone, a 17-ketosteroid of predominantly adrenal origin, continues to attract special interest because of the high level of excretion by adrenal tumor patients with speculation about its role in normal intermediatry metabolism of adrenal cortical steroids. The isolation of urinary ketosteroid conjugates by techniques which will be described initiated a comparison of methods for measuring dehydroepiandrosterone excretion (as a free steroid) with special attention to hydrolytic and isolation techniques and color reactions. For this purpose the 24-hour urinary dehydroepiandrosterone excretion has been studied in five normal men, 25-37 years of age, and five normal women 25-30 years of age.

During a study of hydrolysis of conjugated ketosteroids isolated from butanol extracts of urine by adsorption chromatography on an alumina column as described by Crepy et al. (*Acta endocrinol.* 24:233, 1957), the Allen method for measuring the dehydroepiandrosterone in a mixture of sulfated ketosteroids was found to be far more sensitive and specific when a relatively simple method of hydrolysis of the ketosteroid sulfates by a solvolysis procedure described by Burstein and Lieberman (*J. Biol. Chem.* 233:331, 1958) was used instead of acid and heat in the usual manner. Distinct absorption peaks at 600 mμ were produced with low concentrations of dehydroepiandrosterone.

The Zimmerman reaction was twice as sensitive as the Allen reaction for free dehydroepiandrosterone isolated by paper chromatography (isooctane-85% methanol) after solvolysis of the ketosteroid sulfates obtained from the Crepy column. By the two-step isolation procedure and measurement with the Zimmerman reaction the 24-hour urinary dehydroepiandrosterone excretion for this group of normal adults ranged from 0.3 to 5.5 mg/24 hours. The individual variability of excretion of the 17-ketosteroid sulfates (dehydroepiandrosterone, androsterone, etiocholanolone) will be mentioned.

The Allen method was modified so that it could be used as a simple screening test for dehydroepiandrosterone excretion at low concentrations. A simple butanol extraction of the urine at pH 11 and hydrolysis by solvolysis instead of acid and heat resulted in a marked reduction in interfering substances which permitted the detection of 8μg. of dehydroepiandrosterone in 20 ml. of urine.

### 127. The Production of Type 19S γ-Globulin Antibody by Neonatal Infants in Response to Typhoid Immunization

Richard T. Smith, John James,*
Donald V. Eitzman,* and Billievelyn Miller,*
Florida and University of Texas Southwestern

Existing data indicate marked impairment in the newborn infant's ability to form various antibodies. Perhaps for this reason *active* immunity as a significant factor in the infant's usually successful encounter with his normal bacterial flora has not been evaluated experimentally. The studies to be presented were designed to characterize qualitatively and quantitatively the earliest manifestations of active immunity to enteric microorganisms.

The antibody response to a vaccine containing the organism E. typhosa (0.05 ml. TAB vaccine, Wyeth, intradermally in two sites) was evaluated in more than 150 premature and full-term infants. A prebleeding and weekly or more frequent blood serum samples taken after immunization were tested for the presence of antibody against the O and H antigens of this organism by a tube-agglutination technique in serial twofold dilutions.

One week after injection 46 out of 62 infants who were 0-30 days of age and who had no detectable passively transferred antibody in the preimmunization specimen had developed antibody to the H antigen in 1-10 dilution or higher. The titers varied from 1-10 to 1-640 with the H antigen, but detectable anti-O was present in only one of the sera. At two weeks 51 out of 58 of these infants showed anti-H titers of 1-10 to 1-5,120, and 4 of the older infants in the series had anti-O titers of 1:5. When sorted for age at immunization and birth weight, significant differences were evident neither in the proportion responding nor in the titers at 2 weeks; 28 out of 34 infants in another series immunized between 1 and 3 days of age had detectable anti-H at 2 weeks of age.

Nonimmunized infants with and without passively transferred antibody showed no spontaneously appearing antibody during the first month of life. Passively transferred antibody to H antigen was found in approximately 35% of all infants studied, but with the technique used only 2% had low titers of passive anti-O. Comparison of maternal cord blood titers showed quantitative transfer of anti-H in the more mature infants.

Characterization of the actively produced antibody to the H antigen has revealed that (*a*) the antibody is removed by several absorptions with H antigen; (*b*) on starch block electrophoresis it migrates in most infants studied as a

* By invitation.

$\gamma_1$-globulin; (*c*) ultracentrifuge studies with use of a modification of the Waugh separation cell show that the actively produced antibody is of the 19S type—none is 7S. In contrast the passively transferred anti-H was a $\gamma_2$-globulin of the 7S variety. Preliminary data suggest similar qualitative characteristics of the response to other antigens in this period of life.

These data indicate that the newborn infant has the capacity to respond to immunization by the production of at least one variety of specifically oriented globulin. The cell of origin of this macroglobulin antibody, and its role in natural defense mechanisms is currently being investigated.

### Discussion

DR. HEINZ EICHENWALD, New York: In the past few years we have had the opportunity to study some naturally occurring viral infections in full-term and premature infants. To our surprise we found that these infants were capable of developing a good neutralizing antibody response. Just as Dr. Smith has shown, the appearance of the antibody was in no way related to the infant's age or maturity as measured by birth weight.

I would like to ask Dr. Smith if his 19S γ-globulin is related to the neutralizing antibodies which are developed in children after viral infection.

DR. RALPH WEDGWOOD, Cleveland: Dr. Smith has demonstrated very nicely that infants are capable of responding to an antigenic stimulus at a very early age by the development of antibody. He has previously shown that rabbits at a similar age do *not* respond in this manner to a protein antigen. Indeed under such circumstances rabbits develop a condition that Dr. Smith has termed "obverse immunity," which may be akin to "immunologic tolerance." I wonder whether he would like to go out on a limb and tell us why neonatal rabbits are unable to respond to protein antigens and develop tolerance to the antigen, while infants are capable of responding—in this case to a bacterial antigen.

It is of further interest that he has shown that infants can respond to the "H" antigen of the bacterium, but apparently not to the "O" antigen. Is there something special about the "H" antigen? Or is this lack of response to the "O" antigen related to the immunologic similarities between the somatic ("O") antigens of both bacterial and mammalian cells?

Finally, are the small but immunologically active 7S remnants that he gets from his large 19S macroglobulin by breaking the sulfhydryl bonds just regular "old-fashioned" 7S γ-globulin antibodies; are they immunologically normal γ-globulin, or are they a special species of antibody created artificially by this technique?

DR. ROBERT FOWLER, Melbourne: I would like to ask Dr. Smith what was the youngest child in this series. He mentioned that premature infants can produce these antibodies. How far back in our life history can we trace this antibody response?

DR. ROBERT A. ULSTROM, Minneapolis: I would like to ask a question regarding the ease with which the protein in these antibodies was detected. Do you know what the degradation rate was in this fraction? Is it possible that the easy detectability of this particular fraction may be related at this time of life to a slow turnover rate associated with a decrease in the rate of breakdown of this protein, thus allowing the concentration in the blood to accumulate?

DR. SIDNEY KIBRICK, Boston: Additional evidence is currently accumulating which suggests that the antibody response in the neonate, at least to some antigens, may be better than previously suspected. We recently had an opportunity to study a mother who developed an ECHO 9 infection immediately prior to delivery; also her newborn infant. The baby, presumably infected at birth, developed clinical signs of ECHO 9 disease at several days of age. Mother and infant both developed good titers of neutralizing antibodies to this agent. It is of interest, moreover, that the antibody response in the infant was demonstrable to higher titer than that in the mother.

DR. SMITH: At the present time, Dr. Eichenwald, I do not know the characteristics of neutralizing antibodies. We do have some experiments in progress. We would like to get neonatal serum with neutralizing antibody to examine by our methods for detecting 19S antibodies.

Dr. Wedgwood asked why do the infants form antibody rather than becoming tolerant as occurs when neonatal rabbits receive protein antigens. We have been partially successful in developing a similar experimental model in rabbits by intraperitoneal injection of large amounts of typhoid antigen with resultant agglutinin formation. The same rabbits will develop immunological tolerance to soluble protein antigens. This emphasizes the point which we have made several times; that the nature of the antigen is an important determinant of the animal's response to it.

Different types of antigens are perhaps handled by different cell systems, and different metabolic pathways are involved in directing the response of the animal—either making antibody or inhibiting antibody formation.

With respect to the lower molecular weight fragments which are formed from the macroglobulin by thiol compound treatment, it is not known whether these are simply 7S antibodies or incomplete fragments in this particular case. Data in the literature in the case of the pathological

and normal macroglobulins in adults indicate that the fragments are not classical 7S globulins but have special characteristics. The 19S has a high carbohydrate content. The fragments have more carbohydrate than is found in "normal" 7S globulin. Apparently this carbohydrate is necessary to stabilize macroglobulin. Different from other investigations however, we have found that the fragments *do* retain agglutinin activity.

In response to the gentleman from Melbourne, the youngest infant in which we have a response was 6 days of age—but this is the earliest we have taken a specimen. Most adults have an antibody response to this antigen in four days.

We have no data on the metabolic degradation rate or the turnover rate of this particular type of globulin. It is a matter of great interest, particularly in view of Dr. Ulstrom's own studies indicating that degradation rates do have something to do with the accumulation of antibody globulin in the neonatal period.

Since the integrity of this macroglobulin depends upon disulfide bonds, it is tempting to speculate that in the neonatal infant a deficiency exists in the mechanisms which in adult mammals normally cause the dissolution of disulfide bonds of macroglobulins. Already other profound metabolic differences in the neonatal period and adult life have been demonstrated before this Society, so it is conceivable that this mechanisms could also be impaired.

### 128. Fetal Hormones in Development: Development of the Thyroid Gland

EDNA H. SOBEL, MAX HAMBURGH,* and ROBERT KOBLIN,* Albert Einstein College of Medicine

The high incidence of mental defect in congenitally hypothyroid children remains a serious problem whose solution involves evaluation of the role of maternal and fetal thyroid hormones in fetal development. Because the many variables cannot be controlled in studies of human beings, the rat has been chosen as an experimental animal. In the course of these studies a combination of propylthiouracil and potassium perchlorate was given to intact pregnant rats. Each animal received 0.075% propylthiouracil and 0.75% potassium perchlorate in the drinking water. In both maternal and fetal thyroids marked disorganization of the follicles was found, as well as increased vascularity typical of the response to excess thyrotrophic hormone.

In order to determine whether the effect on the fetal thyroid is induced by maternal or by fetal thyrotrophin, hypophysectomized pregnant animals are being treated with the same combination of drugs. The development of goiters in the absence of the maternal pituitary would indicate that the fetal pituitary produces thyrotrophin.

Some of the hypophysectomized pregnant animals, given the goiterogens, are also being treated with thyroxin or triiodothyronine with the object of detecting transplacental movement of these compounds. Prevention of goiter formation in the fetuses would indicate that maternal thyroid hormone is available to the fetus.

Results thus far indicate that treatment with goiterogen leads to a diminished number of follicles with cells of increased height, as well as disorganization of many of the follicles.

### 129. Infectious Ribonucleic Acid Derived from Enteroviruses

KATHERINE SPRUNT,* WINIFRED M. REDMAN,* and HATTIE E. ALEXANDER, Babies Hospital, New York

Infectious ribonucleic acid (RNA) has been prepared in low titer from infected animal tissues in several different laboratories and in fairly high titer from purified concentrated tissue culture virus preparations in our laboratory. In order to extend these studies and avoid the expense of starting with concentrated virus material, we have applied the phenol-extraction method of Gierer and Schramm to crude unpurified tissue-culture-grown virus. The virus is grown on HeLa-cell monolayers in test tubes in the presence of Scherer's maintenance solution with 10% horse serum. Viruses are harvested and frozen when cytopathic action appears to approach maximum. With use of such crude material, it has been possible to prepare infectious RNA from all of the enteroviruses in which the attempt has been made. This includes two types of poliovirus, two types of Coxsackie Group B virus, and two types of ECHO virus. The infectious material is presumed to be infectious RNA in that activity is totally eliminated by purified ribonuclease and not at all by deoxyribonuclease. In each instance there has been approximately a $10^9$ drop in infectivity titer in the course of the RNA preparation from intact crude virus. The RNA preparations still have a relatively high titer, however, and many can be diluted one-thousand-fold and still regularly produce plaques on HeLa-cell or amnion-cell monolayers.

With use of the above method, several attempts have been made to isolate infectious nucleic acid from vaccinia virus which is presumed to contain deoxyribonucleic acid. These attempts have been unsuccessful.

* By invitation.

### 130. Nephrotic Syndrome Complicated by Renal Tubular Dysfunction

GUNNAR B. STICKLER,* ALVIN B. HAYLES,* MARSCHELLE H. POWER,* and JOHN A. ULRICH,* Mayo Clinic
Introduced by Robert L. Vernier

We have observed two girls with chronic renal insufficiency associated with long-standing nephrotic syndrome. In each instance dwarfism, rickets, and tetany were presenting clinical symptoms, while acidosis, azotemia, hypoproteinemia, proteinuria, glycosuria, and amino-aciduria were the striking laboratory abnormalities. Administration of vitamin D resulted in healing of the rickets. In a metabolic study of one of the children, administration of prednisone was followed by a decrease in proteinuria and amino-aciduria, an increase in the serum protein, and a decrease in the hypercholesterolemia. Glycosuria was unchanged. The addition of vitamin D to intermittent steroid therapy further reduced the amino-aciduria and improved the hydrogen-ion exchange mechanism.

Our paper will report observations made on these patients and discuss the possible role of steroids and vitamin D in the management of children with renal insufficiency of this type. We gained the definite impression that the decrease in proteinuria was primarily responsible for the improvement of tubular function. This supports the hypothesis that prolonged proteinuria will interfere with normal tubular function.

### 131. Temporary Megaloblastosis Associated with Parenteral Iron Therapy in a Case of Iron-Deficiency Anemia

PHILLIP STURGEON, CHARLES BRUBAKER,* and VINCENT ALDO,* University of Southern California

A 16-month-old 20 lb. boy whose diet had consisted entirely of cow's milk was admitted to the hospital with rapid breathing, pallor, slight swelling of the eyelids and minimal enlargement of the liver. The hemoglobin concentration was 1.8 gm., and the erythrocytes were microcytic and hypochromic.

An initial series of hematologic tests established the diagnosis of iron-deficiency anemia. Small blood transfusions increased the hemoglobin concentration during the next 12 hours to 5.8 gm. Respiratory distress was not relieved, and rales developed; oxygen, antibiotics, and digitalis were given. Feedings were restricted exclusively to milk. The patient's condition improved over a three-day period. A second series of tests were taken; parenteral iron, 200 mg., was administered over the ensuing three days. Previous studies on similar cases treated with parenteral iron had shown a beginning reticulocytosis in two to three days (see tabulation above).

Six days after the first injection of 100 mg. of iron reticulocytosis had not developed; a third series of tests was taken; the bone marrow showed marked megaloblastosis. Three days later, with essentially no change in therapy, the bone marrow showed normoblastic erythropoiesis. Two days after this (eleven days after the first injection of iron), reticulocytosis was distinct, and on the thirteenth day it reached a maximum of 18.4%. The milk diet was continued until the 17th day, when a final series of tests was made.

The transient megaloblastosis in this case suggests a temporary relative deficiency in erythrocyte maturation factor(s). It is postulated that the massive availability of iron associated with parenteral administration suddenly permits the rate of erythropoiesis in the severely iron deficient subject to approach its absolute maximum. This maximum is known to be far in excess of that required to maintain equilibrium under normal conditions. Under circumstances of greatly accelerated normoblastic proliferation, relative deficiency of erythropoietic substance(s) could be manifested in megaloblastosis. Similar relationships may explain the reversion to normoblastic erythropoiesis as maturation proceeds and physiologic equilibria are approached.

* By invitation.

| | | Hematologic Observations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Test | Day | Hb. | Retic. | MCV | SI | % Sat. | SC | FEP | Bone Marrow | Therapy |
| 1st | 1 | 1.8 | 5.2 | 63 | 16 | 5 | 37 | 96 | — | Tx., supportive |
| 2d | 3 | 5.8 | 3.5 | 82 | 101 | 37 | 50 | 46 | Normoblastic | I. M. iron, 250 mg., 3 days |
| 3d | 10 | 7.2 | 1.2 | 84 | 400 (post iron dextran) | — | 53 | 50 | Megaloblastic | Milk diet exclus. |
| 4th | 14 | 6.6 | 2.8 | 87 | 52 | 22 | 40 | 89 | Normoblastic | 2 days later, reticulocytes, 18% |
| 5th | 18 | 8.9 | 14.8 | 89 | 75 | 31 | 58 | 177 | — | — |

## 132. Quantitative Estimation of the Diffusion Gradient of Bilirubin in Erythroblastosis Fetalis

GEORGE K. SUMMER,*
University of North Carolina
Introduced by Edward C. Curnen Jr.

After exchange transfusion in jaundiced newborn infants a "rebound" increase of bilirubin in serum is frequently noted. An important factor in the rebound is the variable extravascular reservoir of bilirubin which may diffuse in or out of the plasma. One must hypothesize that a gradient for bilirubin from the tissues to plasma is established based upon a large concentration of bilirubin in the tissues.

This study defines the gradient quantitatively according to Fick's law of diffusion $\frac{dc}{dt}=D\frac{d^2c}{dx^2}$ where $c$ is the concentration in serum, $t$ is time in seconds, $D$ is the diffusion coefficient (the weight of material diffusing across a plane of 1 sq. cm. in area in unit time under a concentration gradient of unity), and $dx$ is the distance the substance moves in time $dt$. The mathematical derivation of the equation where $c_o$ is the membrane concentration and $c$ is the concentration at distance $x$ $c=c_o\left(\sqrt{\frac{1}{\pi Dt}}\right)e^{\frac{-x^2}{4Dt}}$ if $\frac{-x^2}{4Dt}$ is approximately constant reduces to $lnc=ln\frac{c_o}{\sqrt{\pi D}}-\frac{1}{2}lnt-\frac{x^2}{4Dt}$

($K_1$, $K_2$ constants)

$$\log c=K_1-\frac{1}{2}\log t-\frac{K_2}{t}$$

$$\log c+\frac{1}{2}\log t=K_1-\frac{K_2}{t}$$

if the distance $x$ is considered very small. The functions which yield informative data are log $c+\frac{1}{2}$ log $t$ versus the reciprocal of time. (All equations will be derived.)

Data from patients with erythroblastosis fetalis with one, two, three, and four exchange transfusions and patients with physiologic jaundice were tested according to this hypothesis. Patients with two, three, and four exchange transfusions exhibit a uniform time rate of change and membrane concentration which closely approximate values found in physiologic jaundice. Patients requiring only one exchange transfusion show a much slower time rate of change and a significantly lower membrane concentration than those patients with multiple exchange transfusions and physiologic jaundice.

Although many variables have not been considered in analysis of the data, the results suggest that steady-state diffusion relationships can be shown to hold to a certain point in time.

## 133. Toxicity of Chloramphenicol for the Newborn Infant

JAMES M. SUTHERLAND,* ALFRED F. MICHAEL,* ROGER G. GIESEL,* WILLIAM H. KELLER,* and BERNARD A. BEBER,* Cincinnati
Introduced by Frederick N. Silverman

Unexplained deaths of newborn infants during chloramphenicol therapy required more knowledge of the disposition of the drug and its metabolic effects.

1. The acute lethal effect of chloramphenicol to the newborn animal is not limited to the human species. The $LD_{50}$ for newborn rats was found to be one-tenth to one-twentieth of that determined in adult rats.

2. A larger proportion of chloramphenicol circulating in the blood is in an altered and inactive form in infants (37%, range 29%-53%) than in adult subjects (20%, range 2%-38%).

3. A smaller proportion of chloramphenicol is altered in the urine of infants (82%, range 68%-91%) than adults (72.5%).

4. In adult humans and in most animals the main metabolic end product of chloramphenicol is the monoglucuronide. A competitive inhibition between chloramphenicol and endogenous compounds for the coupling enzyme has been suggested. This was not borne out by comparison of serum bilirubins in a group of chloramphenicol-treated infants and in a group of untreated control infants. The serum bilirubin was not consistently related to total blood chloramphenicol or to the proportion of metabolized to unmetabolized drug in the blood. It is of interest that one infant had 32% free and unmetabolized urinary chloramphenicol and a bilirubin of 8.4 mg. % at three days; another infant had only 12.8% unmetabolized chloramphenicol at three days, with a serum bilirubin of 2.1 mg. %.

5. Blood levels of total chloramphenicol determined in infants receiving 100 mg. per kilogram per day, the smaller recommended dosage at the time of the study, demonstrated extremely high blood levels. After 72 hours of therapy the mean level was 95μg. per milliliter (range 71-122).

6. One full-term infant who received 100 mg. per kilogram per day for a period of 72 hours had a blood level of 183μg. per milliliter eight hours after his last dose. The blood level of chloramphenicol was 230μg. per milliliter 76 hours after his last dose and a few hours after the onset of the syndrome associated with chloramphenicol toxicity. Twenty-four hours later the level was 201μg. per milliliter, and 24 hours later the infant died with no satisfactory gross or microscopic explanation for his death. Another 1,250 gm. infant inadvertently received 176 mg. per kilogram per day. Thirty-six hours after the

* By invitation.

onset of therapy the infant developed the typical clinical picture and the drug was discontinued. He died 12 hours later, with a blood level of 237μg. per milliliter.

7. Renal clearance data on chloramphenicol and its metabolites will be presented.

8. The effect of chloramphenicol on in vitro systems will be discussed.

The data suggest that more than one system may be responsible for the peculiar response of the newborn infant to chloramphenicol. Although metabolism of chloramphenicol may be altered in the newborn, the elevated blood levels in infants and particularly those whose deaths are otherwise poorly explained are more likely related to poor excretion primarily of conjugated chloramphenicol.

### 134. Evaluation of a Latex-Agglutination Screening Test for γ-Reacting Globulin in Serum

JANE F. TAYLOR,* and WARREN E. WHEELER,
Children's Hospital, Columbus

We have developed a screening test for agammaglobulinemia based upon inhibition by selected dilutions of patients' serum of the reaction between Rh-sensitized red cells and anti-human-globulin serum (Coombs serum). While carrying out evaluations of this method, Dr. Roy Fisk supplied us with a commercial latex reagent for this purpose which proved to be superior.

The reagent consists of polystyrene latex particles coated with antibody produced in rabbits against human γ-globulin. Such particles agglutinate in optimal quantities of human γ-globulin, and the reactions are observed on a slide with the naked eye. By using three easily prepared dilutions of a drop of serum, the screening test readily differentiates sera containing less than 25 mg. % of γ-globulin from those containing between 50 and 200 mg. % and from normal sera. Comparison with paper electrophoretinograms shows the superiority of this method over the data provided by electrophoresis in sera of very low γ-globulin concentrations. However, this test is not sufficiently sensitive to follow changes in γ-globulin of less than 200 mg. %. By assigning scores of 1+ to 3+ for degrees of agglutination, by utilizing the tendency to prozone in high concentrations of γ-globulin, and by more precise diluting techniques, somewhat greater accuracy may be achieved. Even with this improvement in accuracy the test is still not satisfactory for following the destruction of administered γ-globulin to patients with agammaglobulinemia.

The reagent is standardized by observing the reactions of the reagent with selected dilutions of electrophoretically pure γ-globulin or of serum of known γ-globulin concentration. The results are quite reduplicable, and repeated testing of the same sera gives consistent assignment of the sera to one of four categories, agammaglobulinemia, hypogammaglobulinemia, normal, or hypergammaglobulinemia. The reagent is stable, results remaining constant over the four months period during which the evaluation studies were conducted. These data support the contention that the latex agglutination test is satisfactory as a screening test for clinical use and is more practical than the Coombs inhibition test for the detection of patients with agammaglobulinemia and hypergammaglobulinemia.

### 135. The Effect of Immediate Versus Delayed Clamping of the Umbilical Cord on Mortality and Respiratory Rates of the Newborn Infant

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and Elizabeth Steel Magee Hospital

Introduced by Robert Klein

Much has been written about the beneficial effect of the relatively large infusion of blood received

| | Premature | | | Term | | |
|---|---|---|---|---|---|---|
| | Early-Clamped | Late-Clamped | *P* | Early-Clamped | Late-Clamped | *P* |
| No. Enrolled | 199 | 141 | | 93 | 89 | |
| No. Deaths (1st wk.) | 19 | 25 (17.0) * | | 0 | 0 | |
| Mortality, % | 9.6 | 17.7 (12.1)* | >0.3 ($X^2$=1.00) | 0 | 0 | |
| Survivors & Deaths | | | | | | |
| % Infants with Tachypnea | 17.7 | 26.0 * | <0.02, >0.01 ($X^2$=6.71) | -- | -- | |
| % Infants with Tachypnea & retractions | 7.3 | 13.9 * | <0.01 ($X^2$=8.66) | -- | -- | |
| Survivors Alone | | | | | | |
| % Infants with Tachypnea | 16.2 | 26.2 * | <0.001 ($X^2$=10.00) | 20.8 | 13.7 | <0.01 ($X^2$=9.61) |
| % Infants with Tachypnea & Retractions | 5.8 | 13.5 * | <0.001 ($X^2$=14.18) | 0 | 0 | |

* Adjusted so that distribution of infants by 500 gm. weight groups is identical to early clamped weight group distribution.

* By invitation.

by the newborn infant when delayed clamping of the umbilical cord is carried out. We have, however, been unable to discover a satisfactorily controlled series that compares the clinical course of early- and late-clamped infants. We have reported the findings of normal umbilical vein pressure in newborn infants whose umbilical cords were clamped at the time of delivery and temporarily but significantly elevated umbilical vein pressure in infants whose cords were clamped after a delay of three minutes, during which time the infants were held below the level of the perineum. This suggested to us that the late-clamped infants were overinfused with placental blood and might, under certain circumstances, be harmed by this rapid and relatively large transfusion. The following data were obtained to test this hypothesis.

One hundred eighty-two full-term and three hundred forty premature infants were enrolled in this study during a period of 23 months. All infants were delivered *per vaginam*. Infants whose cords were clamped within 15 seconds of delivery, during which time they were held above the level of the perineum, were considered early-clamped. Infants accepted for the late-clamped group were held below the level of the perineum for at least one minute (the majority for three full minutes) before the cord was clamped. All infants were observed carefully from the time of delivery. Respiratory rates and character of respirations were noted every four hours for the first day, and every eight hours for the second day of life. The nursery management of the two groups was identical. Analysis of various pertinent factors reveals the groups of early-clamped and late-clamped full-term infants to be comparable in all respects. The early- and late-clamped premature infants were likewise comparable in all major respects, although the distribution of infants by 500-gm. weight groups is dissimilar for the two study groups. All mortality and respiratory rates are adjusted in the late-clamped premature group so that the weight group distribution of the late-clamped group is identical to that of the early-clamped group. Mean respiratory rates were calculated for each four-eight hour period of observations for both term and premature infants and each infant's record was then expressed in terms of the number of standard deviations by which it varied from the mean. Results, tabulated below, indicate that the time of clamping of the umbilical cord does not significantly affect first week mortality in this series. Late-clamped premature infants had a significantly higher incidence of tachypnea and of tachypnea with retraction than their early-clamped counterparts. The incidence of tachypnea was, however, significantly higher in early- than in late-clamped full-term infants. (See previous page for tabulation.)

Autopsies were done on 84% of the deaths in this series. Distribution of deaths as to autopsy diagnosis is almost identical in the two groups. Mortality rates were found to be elevated for premature infants breathing in excess of 1.5 S. D. above the mean during the first 48 hours of life.

These data indicate that delayed clamping of the umbilical cord does not improve the clinical course of the newborn infant. It is postulated that increased incidence of neonatal respiratory distress observed in late-clamped premature infants might be related to overtransfusion at birth.

### 136. Concentration of Serum Hexuronides and Their Response to Ingestion of Menthol in the Adult and in the Newborn Infant

P. M. Taylor,* J. H. Wolfson,* D. W. Watson,* and N. H. Bright,* University of Pittsburgh and Elizabeth Steel Magee Hospital
Introduced by Robert Klein

It has been suggested by a number of reported observations that physiological jaundice of the newborn infant results primarily from decreased ability of the infant to conjugate indirect bilirubin with glucuronic acid. The purpose of this study is to assess the relative abilities of the newborn infant and of the adult to perform glucuronidation in vivo.

The concentration of serum hexuronides in 18 adults and 41 3-day-old infants has been determined by the carbazole method of Dische. Daily determinations were made for the first four days of life on six of the full-term infants studied, making a total of 59 separate determinations. Significantly higher values were found in the adults than in the infants: 3.47 mg/100 ml. $\pm 0.62$ (S. D.), and 2.33 mg/100 ml. $\pm 0.46$, respectively ($0.02 > P > 0.01$). The infants' levels do not correlate with maturity, age, or extent of indirect bilirubinemia. Cord-blood concentrations vary from 15%-50% of maternal concentrations. A slight but constant positive artery-vein difference is found in the umbilical cord.

The finding of low levels of serum hexuronides in infants relative to adults indicates lower production of hexuronides by infants than by adults, as urinary excretion of hexuronides has been shown to be lower, both in concentration and in total output per kilogram body weight or per surface area, in infants than adults (Brown, Lacson, and Waters). Low production of hexuronides by the infant could be explained by either (1) diminished quantity of substrates requiring hexuronidation (glucuronidation) or (2) impaired ability of the infant to form glucuronides. Although the first possibility seemed unlikely in face of the excess of unconjugated bilirubin found in the serum during the first few days of life, we attempted to test

* By invitation.

the ability of the infant to form glucuronides by feeding menthol (20 mg. per kilogram of body weight) to 3-day-old infants and to adult controls. Menthol is excreted in the urine as menthyl glucuronide. Adults show a rise in serum hexuronides of 0.8-1.4 mg/100 ml., with the peak elevation occurring 60-90 minutes after ingestion, and rapid excretion of menthyl glucuronide in the urine. Infants respond with a smaller (0.0 to 0.6 mg/100 ml.) and often insignificant rise in serum hexuronide values and with correspondingly reduced urinary excretion of menthyl glucuronide.

These data supplement other recent studies that implicate defective glucuronidation as an important factor in the genesis of physiological jaundice of the newborn.

### 137. Conduction Velocity of Motor Fibers of the Ulnar Nerve in Infants and Children

JUERGEN E. THOMAS, EDWARD H. LAMBERT, and LLOYD E. HARRIS, Mayo Clinic and Foundation
Introduced by Roger L. J. Kennedy

The conduction velocity of motor fibers of the ulnar nerve in the forearm was measured in 146 persons, including 6 premature infants, 42 full-term newborn infants, and 98 children up to the age of 14 years. In premature babies, values as low as 18 meters per second were found. At full-term birth, velocities ranged from 21 to 33 m. per second, with a mean of 28, which is slightly less than half the values obtained in young adults (48 to 72 m. per second, with a mean of 60). At the age of 6 months, the values for a few infants already approached the lower limit of adult values; by the age of one year, the velocity had increased to a mean of 50 m. per second. At 3 years, almost all values were in the lower part of the adult range. At the age of 5 years, the values were essentially the same as those obtained in adults. These data are in harmony with the observations of Rexed and others that the diameter of the nerve fibers of the ventral roots in the newborn is roughly half that of the adult, and that maturation is attained between 2 and 5 years of age. It is of interest that the H-reflex response (a monosynaptic reflex response to stimulation of the ulnar nerve seldom seen in normal adults) was readily elicited by stimulation of the ulnar nerve in most newborns but did not occur after the age of one year. The conduction velocities in the sensory fibers subserving this reflex were similar to those of the motor fibers. The data obtained have been of value in the study of neuromuscular disorders in children, particularly in the diagnosis of chronic polyneuropathy wherein conduction velocities are low.

### 138. Prognostic Value of Serial Electrocardiography in Isolated Ventricular Septal Defects

W. M. THOMPSON JR.,* OMAR SOSA,* and J. F. DAMMANN JR., University of Viriginia

The natural history of patierts with isolated ventricular septal defects spells change. The variable and frequently progressive clinical picture depends upon the relative size and location of the defect and the response of the pulmonary vascular bed to stress. With a ventricular septal defect, the left ventricle is stressed only by the increased volume load due to the left-to-right shunt, while the right ventricle is stressed by a pressure load associated with varying pulmonary vascular resistances and, in the presence of a right-to-left shunt, by an increased volume load as well. In essence, therefore, the natural history may be characterized as the changing work-load balance between the two ventricles. Work-load shifts are reflected in the electrical activity of the heart. Hence, serial electrocardiograms offer a means of evaluating the wide spectrum of clinical patterns.

In a limited number of patients the prognostic value of serial electrocardiograms was evaluated by an analysis of the clinical course, cardiac catheterization data, anatomical findings at operation or postmortem examination, and the degree of pulmonary vascular alteration. Relatively small defects early demonstrated left ventricular preponderance which became less significant with age. Medium-sized defects were associated with decompensation in infancy and demonstrated progressive left ventricular hypertrophy accompanied by varying degrees of right ventricular activity. Large defects with pulmonary hypertension presented as either right or left ventricular preponderance depending upon the volume of shunt but in all instances eventually developed progressive right ventricular hypertrophy. Thus, serial electrocardiograms offer an excellent method of both understanding the natural history of ventricular defects and of aiding the physician in the decision as to when surgical intervention is indicated.

### 139. Infantile Pyknocytosis, a Common Erythrocyte Abnormality of the First Trimester

PHILOMENA TUFFY,* AUDREY K. BROWN, and WOLF W. ZUELZER, Child Research Center and Children's Hospital of Michigan

The existence of a striking morphologic abnormality of the erythrocyte, the burr cell, has hitherto been reported in infants only in connection with a toxic hemolytic anemia associated with Heinz-body formation and in patients with severe renal disease.

* By invitation.

Studies designed originally to elucidate possible mechanisms of hyperbilirubinemia of the newborn have led us to the recognition of the occurrence of burr cells as an age-determined phenomenon characteristic of the first trimester of life. Normal full-term infants regularly show a frequency of 0.3% to 1.8% of these cells as compared with a maximum of 0.3% in adults. Prematures without exposure to drugs characteristically have a frequency in the range of 0.5% to 5.6%.

On the basis of these control data we were able to recognize a spectrum of a hematologic disturbance in which the frequency of burr cells could be roughly correlated with the occurrence of a recognizable hemolytic process. At one end of the spectrum were patients with hyperbilirubinemia and a moderate increase in the number of burr cells; at the other end were patients with a severe hemolytic anemia usually with a marked increase in burr-cell frequency (up to 50%).

The phenomenon was not demonstrably related to exposure to toxic substances, renal or hepatic disease, infection, or intrinsic erythrocyte defects and in all cases proved transient and compatible with survival.

We propose the term "infantile pyknocytosis" for this relatively common condition.

### 140. Withdrawn

### 141. Vectorial Analysis of the Electrocardiogram of the Newborn and Premature in Health and in Disease

CARLOS VALLBONA* and MURDINA M. DESMOND, Baylor University

One hundred seventy-six electrocardiograms obtained in one hundred four infants born at term and in forty-one premature infants were submitted to vectorial analysis. The age of the term infants at the time of the study ranged from 30 minutes to 10 days. The range in the prematures was 4 hours to 40 days.

A quantitative estimation of the direction and magnitude of the vectors of P, QRS, and T was made using the method of Grant. An attempt was made to quantitate the horizontal projection of those vectors according to the method of Simonsen. The tracings were analyzed separately according to the following criteria: (1) duration of pregnancy, (2) clinical condition of the infant at the time of the study, (3) prenatal complications during the last trimester.

A total of 65 infants born at term were considered to be in good health at the time of examination. The results obtained in this group were less variable than anticipated. The mean vector of P had the following values: direction in the frontal plane +55 degrees (S. D. 15 degrees), magnitude 4μv. per second; direction in the horizontal plane (azimuth) +70 degrees, magnitude 4μv. per second. The mean vector of QRS had a direction in the frontal plane of +130 degrees (S. D. 40 degrees) with a magnitude of 6μv. per second; azimuth +110 degrees and magnitude 16μv. per second. The mean vector of T had a direction of +40 degrees in the frontal plane (S. D. 50 degrees) and a magnitude of 14μv. per second; its azimuth was —60 degrees and the magnitude 14μv. per second.

The scalar electrocardiographic values of the healthy newborn infants were found in agreement with the results previously reported by many authors.

The vectorial and scalar values in infants with respiratory distress syndrome in prematures had greater variability. The vector of T was the most variable and susceptible to change depending on the clinical condition and the treatment measures. Serial evaluation of the vectorcardiogram in pathological conditions yielded information which paralleled the clinical status of the patient.

### 142. Studies of the Duration of the Mechanical Systole in the Newborn in Health and in Disease

CARLOS VALLBONA,* MURDINA M. DESMOND, and RICHARD G. CRADDOCK,* Baylor University

The duration of the mechanical systole in 104 infants born at term and in 41 premature infants was computed from simultaneous tracings of the electrocardiogram and the phonocardiogram.

The results obtained in 65 healthy newborn infants were compared to those found in healthy young adult males. The comparison included the differences in heart rate and the duration of the cardiac cycle. The mechanical systole time of the healthy newborn infant was found to be shorter than that observed in the adult at identical heart rates. This shortening of the mechanical systole was not always followed by a parallel shortening of the QT interval of the ECG. The analysis of results obtained previously in adult subjects under different physiological and pathological circumstances and the results of this study suggest that a shortening of the mechanical systole indicates an increased but more efficient metabolic activity of the myocardium necessary for the adaptation to a new environment.

The influence of several pathological circumstatistical parameters for contraction times are was determined from multiple correlation studies which considered several prenatal and neonatal clinical variables. It appears that the administration of some drugs to the mother in the last term of the pregnancy, i. e., Rauwolfia serpentina, chlorpromazine, acetazolamide, is associated with a pro-

* By invitation.

longation of the mechanical systole in the newborn infant. The mean and median values of systole time in the premature infants were much larger than those of healthy newborn infants. The pathological syndrome of respiratory distress was usually accompanied by a prolongation of systole also. Some therapeutic agents, e. g., digitalis and cortisone, appeared to produce a shortening of the mechanical systole in the infants to whom they were administered. Further investigation of this point is needed.

### 143. Soybean Goiter

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A 10-month-old girl, reared on soybean milk since birth, presented with signs of hypothyroidism and goiter of two months' duration. Although soybean flour is a well known goitrogen in animals, a similar action in humans, except for the report of Rawson (*Recent Progress in Hormone Research*, 11:273, 1955), has not been described.

Studies were designed to evaluate the effect of soybean milk on the thyroid function of this infant and on that of eleven healthy adult volunteers. All studies were carried out in an identical manner on two distinct regimens consisting of soybean milk and whole cow's milk in which the latter served as the control situation.

Radioactive iodine kinetics in this infant while on the cow's milk regimen were characteristic of iodine depletion and consisted of an initial rapid thyroidal accumulation of $I^{131}$ followed by a rapid discharge of $I^{131}$ from the thyroid gland. During this period there was an abnormally high rate of conversion of $I^{131}$ into protein-bound $I^{131}$. On a soybean milk diet exclusively, the infant had an $I^{131}$ uptake curve similar to the one noted above, although a significantly smaller percentage of the administered dose was trapped by the thyroid. The conversion of $I^{131}$ into protein-bound $I^{131}$ was completely inhibited during this period. The patient was treated with one drop of Lugol's solution daily for four days, and the soybean milk diet was replaced by a normal diet for her age. Four months later, she was euthyroid clinically and all thyroid function studies were normal.

In the 11 healthy adults studied there was no significant change in $I^{131}$ uptake on the two different milk regimens, and in 9 of the 11 subjects there was no difference in the protein-bound $I^{131}$ fractions in the blood. Two of the adults, however, with high protein-bound $I^{131}$ fractions on whole cow's milk appeared to have had a drastic decrease (100% and 50%, respectively) in this fraction while receiving soybean milk exclusively.

These studies imply the presence of a goitrogen in soybean milk which in suspectible persons modifies thyroid metabolism. Iodine depletion may be the ultimate result of the action of this goitrogen. Conclusive evidence is not available, however, to permit a definition of the specific mechanism of action.

### 144. Renal Biopsy Studies of the Acute Exacerbation in Glomerulonephritis

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University of Minnesota

The sudden onset of an illness characterized by hematuria, hypertension, and edema in a previously "well" child usually suggests the clinical diagnosis of acute glomerulonephritis. The latter disease has been shown to be caused by certain types of Group A $\beta$-hemolytic streptococci and is associated with complete recovery in most children. However, there is considerable disagreement in the literature and among physicians with regard to the outcome of this disease. We believe that the disagreement stems from the inadequacy of available clinical diagnostic methods which frequently do not permit a conclusive diagnosis and prognosis to be made, and which are occasionally shown in the course of time to have been misleading.

In a study of 30 children with glomerulonephritis, we have attempted to correlate the clinical, bacteriologic, immunologic, and pathologic features of each case with the patient's ultimate course and with the serial pathology of the disease as revealed by renal biopsy. Six patients have been studied in whom the history and the available clinical diagnostic methods suggested a diagnosis of acute glomerulonephritis but in whom the existence of chronic renal disease was indicated by examination of renal biopsy specimens. The common pathologic characteristic of these specimens has been the presence of severe fibrotic glomerular damage and extensive tubular atrophy, associated with an acute glomerulitis in which a prominent polymorphonuclear infiltration of the glomerulus was often noted. The extensive chronic changes observed in the biopsy of each case indicated that an unrecognized pathologic process must have been present prior to the onset of the acute symptomatology, two-four weeks before the biopsy.

The etiology of the acute exacerbation has been studied by appropriate cultures and a search for antibodies against various streptococcal antigens (type-specific antibody, antistreptolysin O, antidesoxyribonuclease). The data show that the acute exacerbation of chronic glomerulonephritis may be initiated by infection with both nephritogenic and non-nephritogenic strains of streptococci, as well as by a variety of nonspecific infections. Thus, the differential diagnosis of acute exacerbations of

* By invitation.

chronic nephritis versus acute glomerulonephritis is complicated by the fact that acute exacerbations of chronic glomerulonephritis may be produced by nephritogenic strains of streptococci. It also seems likely that in some of our cases true acute glomerulonephritis has occurred in patients having preexisting chronic glomerulonephritis.

Our present experience suggests that a partial explanation for the controversy with regard to the natural history of acute glomerulonephritis may arise because it is often so difficult to differentiate the acute exacerbation of the chronic disease from acute poststreptococcal glomerulonephritis. Our observations indicate that the differentiation can often be made by study of percutaneous renal biopsy.

### 145. Metabolism of Human Fetal Adrenals.

Dorothy B. Villee,* Janet Loring, and Claude A. Villee,* Harvard
Introduced by Nathan B. Talbot

This report presents data relating to several aspects of intermediary metabolism in the human fetal adrenal and their relation to steroid biosynthesis. At a previous meeting we reported the presence of 17, 21, and 11 hydroxylases in the fetal adrenal from very early in gestation. The enzyme activities were greatly enhanced by the addition to the in vitro system of TPN and glucose-6-$PO_4$ (thus providing TPNH).

Human fetuses were obtained at therapeutic abortions in Stockholm through Prof. John Lind. The adrenals were removed and tissue slices made with a Stadie-Riggs microtome. Slices were incubated in Warburg vessels in 3 ml. medium containing alternately glucose-1-$C^{14}$ and glucose-6-$C^{14}$ and a buffer of 40$\mu$M. K, 80$\mu$M. Na, 10$\mu$M. Mg, 100$\mu$M. Cl, and 40$\mu$M. $PO_4$, with an initial pH of 6.9. Measurements were made of oxygen consumption, $CO_2$ production, glucose produced (by isotope dilution), lactate formed, and the amounts of $CO_2$ and lipid formed from labeled glucose.

The fetal adrenal has an average oxygen consumption of 22$\mu$M. per gram of adrenal per hour (range: 16-33) and a $CO_2$ production of 42$\mu$M. per gram of adrenal per hour (range: 21-81), giving an average R. Q. of 1.9. Such an R. Q. is consistent with a high rate of lipid or steroid synthesis. The amount of labeled lipid synthesized from glucose-1-$C^{14}$ and glucose-6-$C^{14}$ was 0.137 and 0.127$\mu$M., respectively; essentially the same for both substrates. Thus the two carbon fragments for lipid synthesis would seem to be generated via the glycolytic cycle alone.

By determining the amounts of labeled $CO_2$ from glucose labeled in either position 1 or 6 of the molecule, it is possible to obtain an estimate of the fraction of glucose metabolized via the glycolytic pathway compared with the amount metabolized via the hexose monophosphate shunt. Our results showed that the fetal adrenal has a G6/G1 ratio of 0.056 as compared with ratios of 0.42, 1.0, 0.37, and 0.93 for fetal liver, lung, heart, and cerebral cortex, respectively. Thus the lung has essentially no shunt activity. The liver has an appreciable activity, and the adrenal has by far the most active hexose monophosphate shunt of any fetal tissue thus far examined.

For comparison, a similar experiment was performed using adrenal tumor tissue from a 6-year-old boy with hypertension and virilization. The pathological analysis revealed adenoma. Here again a low G6/G1 ratio was found (0.048/0.55, or 0.087) indicating a very active hexose monophosphate shunt. The amount of labeled lipid from either substrate was again essentially the same. The R. Q. was 1.9. In all parameters tested the tumor did not differ significantly from fetal adrenal tissue.

In the fetal adrenal, it would seem that the majority of glucose is metabolized by way of the hexose monophosphate shunt. Since the first two steps in this pathway convert the cofactor TPN to TPNH, the adrenal is provided with an active TPNH generating system. TPNH is known to be a necessary cofactor for steroid hydroxylations. Our studies on the steroid hydroxylases reveal the presence of these enzymes in the fetal adrenal and their dependence on TPNH for maximum activity. This fact, coupled with the very active hexose monophosphate shunt reported here, provides evidence for the probable ability of the fetal adrenal to convert progesterone to hydrocortisone in vivo.

### 146. Simultaneous Right and Left Heart Catheterization Employing Retrograde Arterial Catheterization in the Investigation of Congenital Cardiovascular Malformations of Infants and Children

Peter Vlad* and Edward C. Lambert,*
University of Buffalo
Introduced by Mitchell I. Rubin

The need for and value of left heart catheterization in the diagnosis of congenital cardiac defects is becoming established. The four approaches to the left heart available are (1) the transbronchial, (2) the anterior thoracic, (3) the posterior transthoracic, and (4) the retrograde arterial. Because of assumed hazards and limitations the last of these has been the least used. There are no systematic evaluations of this retrograde technique in the pediatric age group. Data with respect to its applicability to congenital cardiovascular malformations are scarce. This report presents a systematic study of the value, limitations, and safety of this technique.

* By invitation.

The method employed involves the introduction of a cardiac catheter into the right brachial or femoral artery. Passage through the aortic valve was usually accomplished by means of a looped tip. Simultaneous right heart catheterization was performed by the conventional method.

Eighty-eight patients with various types of cardiovascular malformations were studied in this fashion. Their ages ranged from 5 weeks to 17 years. One-third of them were infants.

Seventeen different major cardiac defects were included. There was a high incidence (29%) of hemodynamically important second abnormalities.

The procedure was successful in 77 out of 88 patients. Some of the failures occurred in the early cases, and we now feel that many would now be avoided. In four instances, the failure of the catheter to pass through the aortic valve appeared to be due to severe aortic stenosis. (However, it was possible to enter the left ventricle in three other cases of aortic stenosis.)

The left atrium was entered from the left ventricle in 18 out of 29 cases (61%) where an attempt was made to enter this chamber.

Diagnostically, this procedure proved to be of special value in that it allows simultaneous pressure determinations on both sides of valves and septa, multiple dye-dilution-curve determinations across the cardiac septa and valves, and selective angiocardiography of the left heart and aorta. Examples of each will be demonstrated.

The chief advantages of retrograde catheterization as opposed to the other approaches to the left heart are (1) convenience of access, (2) less risk of complications due to the fact that neither bronchoscopy nor thoracic or cardiac puncture are involved, (3) the fact that it is an essentially painless procedure requiring only sedation and local anesthesia. The chief limitation is that it is probably not applicable in severe valvular aortic stenosis.

Inherent potential hazards include myocardial ischemia resulting from obstruction of the coronary vessels by the catheter, damage to aortic cusps, and, as in any cardiac catheterization, serious arrhythmias. These dangers proved to be no problem in the present series. No evidence of injury to the heart was seen at postmortem examination in 13 cases. Only death, which occurred six days after catheterization, in an infant with a single ventricle and severe coarctation of the aorta might be ascribed to the method, inasmuch as autopsy revealed a thrombosis of the left renal artery. The only morbidity consisted in transitory asystole which occurred in one child immediately after the injection of contrast medium in the left ventricle. Complete and rapid recovery followed manual stimulation of the chest wall.

As a result of these studies, it appears that simultaneous catheterization of the right and left heart employing the technique of retrograde arterial catheterization is an effective, practical, and reasonably safe diagnostic procedure of special value in detecting and evaluating many congenital cardiovascular malformations other than severe valvular aortic stenosis.

## Discussion

DR. RICHARD L. FOWLER, New Orleans: Dr. Vlad has explored the possibilities of this technique extremely well and has offered some beautiful illustrations of the ways in which it can be used.

I am sure Dr. Vlad is aware of the early basic work done on this technique by a number of investigators, particularly the Mexicans, seven or eight years ago. Despite this initial work most of the laboratories in this country have been hesitant to adopt this technique because of rumors of many disastrous results, including fatal arrhythmias, severe damage to the aortic valve cusps, and coronary damage.

In view of these rumors, when our group became interested in this technique a little more than a year ago, we felt that we had to prove with animal experimentation that the technique was safe. We catheterized 20 dogs by the retrograde arterial techniques, passing the catheter across the aortic valve into the left ventricle two or more times on most of the animals. The looped tip method described by Dr. Vlad was used on some occasions, but more frequently the catheter was passed without a loop directly into the left ventricle. Damage to the aortic valve was minimal in all cases and consisted of a few subintimal hemorrhages. In no instance was the valve rendered insufficient, and no arrhythmias were provoked other than occasional extrasystoles. When considerable manipulation of the catheter was carried out in the left ventricle in an effort to pass the catheter through the mitral valve into the left atrium, scattered subendocardial petechiae and ecchymoses were produced, particularly if a relatively large or stiff catheter was utilized. The hemorrhages were actually no more severe than those encountered in the right ventricle from simultaneous catheterization of this chamber in the usual fashion. However, in view of this experience we suspect that it is wise to use a relatively small and soft catheter to pass across the aortic valve and would like to have Dr. Vlad's comments upon this point.

The one other potential hazard that we encountered during our studies was the frequent passage of the catheter into a coronary artery. Both the passage of the catheter itself and, particularly, injection of dye into the coronary might easily prove to be of serious consequence, and as

a matter of fact this has happened according to word-of-mouth reports. I should appreciate Dr. Vlad's comments concerning this hazard.

With these reservations, we feel that our animal work confirms Dr. Vlad's contentions that this technique is, relatively speaking, a safe and effective procedure.

DR. GLEN G. CAYLER, Oklahoma City: Although the dangers and technical difficulties of retrograde left ventricular catheterization are apparently minimal, I believe that the added risks are sufficient that a retrograde left-sided procedure should not be done routinely with right-sided catheterizations but should be limited to use in patients with left-sided lesions, such as aortic valve disease, and in complicated cases in which the right-sided procedure has not provided adequate information. Necessary left-sided catheterization data can often be obtained during right-sided catheterization by passing the catheter across a patent foramen ovale, atrial defect, or as Dr. Vlad illustrated, through an interventricular septal defect. The high per cent of failure—50%—of retrograde left ventricular catheterizations in patients with aortic stenosis limits the usefulness of this procedure for this anomaly and in many cases necessitates a further procedure to complete the evaluation.

DR. BESSIE L. LENDRUM, Syracuse, N.Y.: In studying mitral insufficiency by selective angiocardiography of the left heart and aorta, were contrast medium injections made into the left ventricle during systole, during diastole, or at random? I ask this because it has been shown in normal dogs that injections made during diastole resulted in marked flow of dye into the left atrium, whereas injections made during systole resulted in little dye entering the left atrium. Thus, it becomes important in assessing the presence of mitral insufficiency from reflux of dye into the left atrium to know during which phase of the cardiac cycle the contrast medium was injected.

DR. VLAD: We are aware of the early work of Zimmerman and of Lemon-Lason and their colleagues, who in 1950 reported a small series of cases done chiefly in order to secure intracardiac electrocardiograms from the left ventricle. Zimmerman and his group reported one death in a series of 11 cases.

With respect to the aortic, and, it should be added, mitral valves, careful autopsy examination of the hearts of 12 patients revealed no evidence of damage to the endocardium or valves. With the one exception mentioned in the abstract, these patients died either at surgery or from the underlying heart disease one week to three months after catheterization.

On two occasions we entered the coronary arteries inadvertently and produced S-T segment changes indicating myocardial ischemia as well as a drop in pressure. These disappeared rapidly following withdrawal of the catheter. We agree that this is a real potential hazard of this technique. It is an important reason for continuous monitoring by a physician of both the ECG and the pressures throughout the procedure. There is no question that there are dangers to this approach. However, on the basis of our present experience with 100 cases, they do not appear to be any greater than those encountered with conventional right heart catheterization.

We agree with Dr. Fowler that it is preferable to use a light (thin-wall) rather soft catheter. The sizes we have employed have ranged from No. 5F to No. 7. For the most part, we use a No. 6F. A suitable curve at the tip is of help. Of course, if aortic stenosis is present, a straight tip may be preferable.

In answer to Dr. Lendrum's question, we did not attempt to correlate the injection of contrast media with the cardiac cycle. The automatic injection through the catheter almost invariably occupied at least two heart beats. The films were taken at a rate of six per second.

### 147. Normal and Abnormal Muscle Contraction Time in Infancy and Childhood

RICHARD L. WALL,* Brooke Army Hospital
Introduced by Leo J. Geppert

Clinicians have long recognized a correlation between the speed of muscle reflexes and the level of thyroid function. Previous attempts to graphically record reflex muscle contraction have been hindered by inadequate or cumbersome instrumentation, and the records obtained did not separate, with precision, contraction from relaxation. Further, no reference has been found in the literature concerning quantitative graphic registration of reflex responses in infancy.

Utilizing a new device, the kinemometer, developed at Brooke Army Hospital, muscle reflexes of infants and children were recorded with a standard EKG. The curve produced is a diphasic curve that records the speed, duration, and direction of muscle movement. With this refinement I have found, contrary to usual clinical teaching, that muscle *contraction* and not *relaxation* correlates best with a child's thyroid status and peripheral neuromuscular development.

A random sample of 253 normal children were studied ranging in age from 1 day to 14 years. It was found that there are basically two contraction patterns, the infantile and the adult. The infantile pattern obtains from 1 day to 11 months, with transition to the adult pattern generally between 11 to 13 months. Early or late transition appears to

* By invitation.

correlate clinically with accelerated or "slow" peripheral neuromuscular development.

Statistical analysis revealed a normal distribution of reflex contraction times in all groups. The other statistical parameters for contraction times are shown in the tabulation below.

| Age Group | No. | Mean Contraction Time, Msec. | Standard Deviation |
|---|---|---|---|
| 1 day-11 mo. | 157 | 125.6 | 11.8 |
| 13 mo.-14 yr. | 61 | 180.7 | 11.5 |
| 11-13 mo. | 35 | 166.9 | 25.3 |

Hypothyroid and hyperthyroid children had muscle contraction times that consistently fell outside the 5%-95% confidence limits for their ages. Hyperthyroid children had *decreased* contraction times, whereas hypothyroid children had *increased* contraction times. When placed on therapy, there was a progressive return of the contraction time to normal, better enabling the clinician to control and alter therapy as indicated.

In summary, the normal muscle contraction time has been defined throughout the pediatric age range. By use of the kinemometer, reflex muscle testing as a routine office procedure was possible, and I have been able to objectively record reflex responses which appear to correlate well with thyroid status and peripheral neuromuscular development in infants and children.

Charts, graphs, typical tracings, and instrumentation technique will be presented.

### 148. Prevalence of Poliovirus Infection in a Partially Immunized School Population

PAUL F. WEHRLE, ORY CARBONARO,* and RALPH REICHERT,* State University of New York, Syracuse

Susceptibility of immunized children to poliovirus I infection was studied in a group of 75 summer-school pupils after the occurrence of two cases of paralytic poliomyelitis in their classmates. Since the entire group met in a single large room for informal morning classes and mingled freely on the playground in the afternoons, considerable opportunity existed for an effective and relatively uniform natural exposure. Only two-thirds of the group had been completely immunized (three Salk-type injections), while 18 had either none or a single immunization. These children represented 42 families living in a small community in central New York.

Initial and final sera, throat swabs twice weekly, and weekly stool samples were collected from all children and 24 sibling household contacts who did not attend school. Poliovirus isolation was in trypsinized cultures of monkey kidney cells.

Poliovirus I was isolated from half (33 of 75) the children. Although the infection rate was higher among those with a history of none or only one previous immunization (12 of 18, or 67%) than in those with either three or four injections (17 of 47, or 36%), the higher infection rate appeared primarily among those of one age group who had received a single immunization. Recovery of poliovirus from the pharynx was achieved in nearly 50% of infected children. The frequency of pharyngeal recovery did not appear to be definitely influenced by either initial neutralizing antibody titer or number of immunizations given prior to the study.

The majority of 15 sibling contacts of infected school children harbored poliovirus I without regard to the immunization status of either the infected school child or the sibling himself. All six triply immunized siblings in household contacts with various triply immunized infected children harbored poliovirus.

Although some decrease in the rate of infection may be present, there seems to be little doubt that the completely immunized school child can become infected with poliovirus I and may serve as a source of the infection for household contacts.

### 149. Dehydration, Starvation, and Trauma: Their Effects on Body Composition

WILLIAM B. WEIL JR. and ANNE TAYLOR,* Western Reserve

Destruction of cells with release of the cell water has been considered one mechanism for economy of extracellular fluid in dehydration. However, if the solute concentration of the cell water is equal to or greater than the solute concentration produced in the urine, no net saving of extracellular fluid could occur, unless hypertonicity of body fluids supervened. The present study was designed to test by direct body analysis whether or not intracellular fluid loss can diminish extracellular loss during dehydration.

Three groups of 10 200-gm. rats were studied. Group I served as controls for whole-body analysis. Groups II and III were caged individually without access to food or water. On the second day of the study Group II animals had a tourniquet applied to one hind leg for two hours. Group II and III animals were killed on the sixth day; at this time they had lost one-third of their body weight.

The whole bodies were analyzed for fat, water, collagen and noncollagen nitrogen, ash, sodium, potassium, chloride, calcium, phosphorus, and magnesium. Blood serum was analyzed for the inorganic elements, and urea nitrogen and hematocrits were determined on the blood.

* By invitation.

In the process of dehydration and starvation the rats in both groups lost 75% of their body fat, 34% of their body water, and 17% of their fat-free dry solids. The traumatized group had a smaller rise in hematocrit (23% vs. 33%), less loss of sodium (20% vs. 25%) and of chloride (24% vs. 27%) than the nontraumatized group. The loss of potassium was somewhat greater in the traumatized group (35% vs. 32%). In both groups the loss of potassium was approximately twice that expected from the loss of protein (18.6% and 18.1% in the two groups, respectively).

Both groups demonstrate a greater loss of intracellular than of extracellular fluid, and the loss of intracellular fluid was greater than the loss of cellular tissue. Although not all the differences between the two experimental groups were statistically significant, the changes were all in the direction which suggests that the increased loss of intracellular fluid in the traumatized group did lead to a decreased loss or relative economy of extracellular fluid without significant hypertonicity.

### 150. The Effect of Triiodothyronine on the Serum Bilirubin in the Newborn

M. C. Westphal,* T. R. Boggs Jr., W. R. Eberlein, and A. M. Bongiovanni,
University of Pennsylvania

The observation of Christensen (*Acta pediat.* 45:367, 1956), that prolonged neonatal jaundice may be associated with cretinism, suggested a study of the effect of a thyroxin-like substance on the serum bilirubin in the newborn.

One hundred newborns were given 25μg. of l-triiodothyronine daily for the first 4 days of life. Serum bilirubin levels were determined on each infant at 24-48 hours of age and 72-96 hours of age. Similar determinations were made on 100 controls. In the test group, 43 infants showed a rise in bilirubin during the interval between the two determinations and 57 infants given the drug showed a decrease or no change in serum bilirubin. In the control group, 64 infants developed a higher bilirubin during the period between the two determinations and 36 infants failed to show such a rise. ($\chi^2$ 8.86, $P<0.005$.) The average difference between the first and second determinations in the test group was 0.45±2.21 mg. %, and the average difference between the two specimens in the control group was 1.57±2.56 mg. %. ($t=6.21$, $P<0.01$. No evidence of difference in variability response.)

These results indicate that the administration of l-triiodothyronine tends to depress the bilirubin level in the newborn during the first 4 days of life.

There was no evidence of bilirubin encephalopathy in any of the infants included in this study. However, the report of Harris et al. (*Pediatrics* 21:875, 1958), describing kernicterus associated with low concentrations of bilirubin in prematures on prophylactic Gantrisin, suggests that a substance which lowers the serum bilirubin may at the same time potentiate its neurotoxic effects. Therefore, we would not recommend the administration of thyroid substance to the newborn in an attempt to control neonatal hyperbilirubinemia, at least until the mechanism of action is clarified.

### 151. A Rapid Test for the Diagnosis of Lead Poisoning

Jo Anne Whitaker* and Teresa Vietti,*
The University of Texas Southwestern
Introduced by E. L. Pratt

A simple and rapid diagnostic test for lead poisoning is desirable now that an at least partially effective treatment is available.

A single drop of blood examined as a wet preparation under dark field using a mercury-arc source and appropriate filters reveals a purplish red fluorescence of every erythrocyte when the patient has lead poisoning. Twelve recent cases have demonstrated these findings.

Our experience with six cases in which all the requisite data were available showed that fluorescence of the red cells was an easier, more reliable, and more rapid criteria for diagnosis than other more commonly used clinical signs or simple laboratory tests.

In addition, the degree of erythrocyte fluorescence proved to be useful in determining the adequacy of treatment and the need for retreatment of patients with lead poisoning.

A similar examination of erythrocytes from 75 patients with a variety of nonhematologic illnesses, 45 with iron-deficiency anemia and 15 with acute hemolytic anemias failed to show as marked fluorescence in any condition; however, slight to moderate fluorescence was seen in some cases of iron-deficiency, hemolytic anemia, etc.

A discussion of the probable mechanisms of the fluorescence observed and its correlation with previous chemical determinations of erythrocyte porphyrins in a variety of conditions will be presented.

### 152. Studies on Lipids in Infancy: Maternal-Infant Differences and the Effects of Human and Cow's Milk Feeding

Calvin Woodruff and John G. Coniglio,*
Vanderbilt

Determinations of the total fatty acids, phospholipids, free and total cholsterol, polyunsaturated fatty acids (determined by alkaline isomerization),

* By invitation.

tocopherols, carotene, vitamin A, and lipoproteins were made on postprandial serum from mothers at term and their newborn infants before the first feeding. The unfed newborn infants had lower values than their mothers for all the lipids studied. Data from 30 mothers and 30 infants are as follows:

| | 30 Mothers at Term | 30 Unfed Newborn Infants |
|---|---|---|
| Vitamin A, I. U. % | 227. | 85. |
| Tocopherols, mg. % | 1.91 | 0.46 |
| Carotene, μg. % | 156. | 24. |
| Total fatty acids, mg. % | 437. | 217. |
| Phospholipid, mg. % | 206. | 91. |
| Total cholesterol, mg. % | 267. | 78. |
| Free cholesterol, mg. % | 84. | 25. |
| Polyunsaturated fatty acids as % of total fatty acids | | |
| Dienoic, % | 19.22 | 3.60 |
| Trienoic, % | 1.16 | 0.86 |
| Tetraenoic, % | 4.33 | 4.82 |
| Pentaenoic, % | 0.61 | 0.65 |
| Hexaenoic, % | 0.84 | 1.00 |

Thirty infants were fed an evaporated milk formula supplemented only with ascorbic acid for three months. Another group was breast-fed and received only vitamin D supplementation. The evaporated milk was all from a single commercial lot of known analysis. Breast milk, serum samples, and dietary histories were obtained from the nursing mothers at six weeks and three months postpartum.

The breast-fed infants had significantly higher concentrations of total fatty acids, free and total cholesterol, dienoic and tetraenoic fatty acids, tocopherols, and carotene than those fed cow's milk. Lower values for trienoic and pentaenoic acids were found in the breast-fed group. No differences were apparent in vitamin A or phospholipid concentrations.

The significance of these findings in infant nutrition will be discussed.

### 153. Maturation and Localization of Glutaminase and Carbonic Anhydrase Activity in Rat and Human Kidney

SUMNER J. YAFFE,* Harvard

Introduced by Charles A. Janeway

The well-known functional immaturity of the newborn kidney prompted an investigation of the activities of the two enzymes most concerned with renal regulation of acid-base economy. Glutaminase, by catalyzing the deamination of glutamine, accounts for the major portion of ammonia formed by the kidney. Carbonic anhydrase accelerates the hydration of carbon dioxide to form carbonic acid, which serves as the source of hydrogen ions for acidification of the urine.

The newborn rat possesses about 50% of the adult level of these two enzymes' activity on the basis of dry weight, as assayed in kidney cortex homogenate. There is a rapid increase in activity between 20 to 30 days of age to levels approaching those of the adult. Attempts to stimulate the activity of glutaminase in the newborn animal, by tube-feeding ammonium chloride to the pregnant or nursing rat results in no change in the low level of activity in the newborn, whereas the mother's activity increases threefold. This could suggest that there is a real deficiency in the actual amount of enzyme present.

The human kidney appears to follow the same pattern of maturation of enzymatic activity as in the rat, with normal levels of activity being attained at about 3 to 4 months of age.

In addition to carbonic anhydrase activity in the cortex, significant activity was found in the papilla of the rat kidney and approximates 80% of cortical activity. This provides a biochemical basis for the recent physiological evidence which appears to place the site of urinary acidification in the collecting ducts as well as in the distal tubule.

### 154. Enzyme Studies in Renal Tubular Acidosis

SUMNER J. YAFFE,* JOHN M. CRAIG, and FRANCIS X. FELLERS,* Boston

Since the original report by Butler and the metabolic studies of Albright, the basic defect in the syndrome of renal tubular acidosis has remained obscure. The primary fault appears to lie in the kidney with a failure of the usual mechanisms of urine acidification. The similarity of the syndrome to the metabolic effects occurring after administration of carbonic anhydrase inhibitors has suggested lack of the enzyme, carbonic anhydrase, as an etiological factor, although no reports of assays of enzymatic activity have hitherto appeared. The present report concerns enzyme activities in a biopsy specimen obtained from a patient with an abnormality in urinary acidification.

The subject under investigation is a 4-year-old girl who first presented because of bilateral nephrolithiasis. Metabolic studies revealed hyperchloremic acidosis with an alkaline urine, good urinary concentrating ability, and normal glomerular filtration rate as determined by endogenous creatinine clearances. Acid-loading with ammonium chloride resulted in an increase in ammonia excretion from basal levels of 20 mEq. per day to 55 mEq. per day. The urinary pH decreased only slightly from 7.16 to 6.22. Titratable acidity increased six- to eightfold after oral administration of neutral phosphate buffer. Despite a low calcium intake, there

* By invitation.

was significant hypercalcuria. In addition, complete metabolic balance studies were carried out, and the results of these will be presented.

Renal biopsy was obtained, and histological examination disclosed minimal alterations in architecture with clumping of mitochondria and loss of the brush border in the cells of the proximal convoluted tubule.

Enzyme studies revealed normal activities of carbonic anhydrase and glutaminase. Because of the morphological abnormality of the mitochondria the activity of several enzymes of the Krebs tricarboxylic acid cycle was assayed histochemically. Activities of succinic dehydrogenase and DPN-linked diaphorase were normal, but activity of TPN diaphorase, which is linked to isocitric dehydrogenase, appears to be deficient. These findings suggest that the inability of the tubular cell to maintain a hydrogen ion gradient is related to an intracellular defect in energy metabolism and not to a defect in the enzymes concerned with the formation of hydrogen ion or ammonia.

## Discussion

Dr. Wallace W. McCrory, Iowa City: It is my understanding that patients whom one considers to have renal tubular acidosis usually demonstrate persistently low bicarbonate and low PH and fail to show much, if any, increase in urinary ammonia when given an acid load. In this sense this patient is not typical. You apparently did observe a substantial rise in urinary ammonia excretion following the administration of an ammonium chloride load. What other evidence (serum bicarbonate and PH before and after ammonium chloride loads) do you have that provides some evidence of the ability of this patient to resist exaggeration of acidosis in response to this load? It would also be unusual in my experience to find that there is preservation of normal urinary concentration ability with this defect. I wonder if you might be dealing with a patient with a mild defect in acidification.

Dr. Neil N. Litman, Los Angeles: In the past year we have undertaken a study of renal biopsy with light and electron microscopy in approximately 40 children with a variety of renal diseases. Included in this study is a child with familial Fanconi's syndrome with metabolic acidosis of a similar nature to that demonstrated by the patient you described. The alterations in the tubules seen with the electron microscope seemed most striking. The major abnormalities seemed to be in the proximal tubules. The mitochondria were decreased in size and density and the cristae were not clearly visible. The brush border was distorted. In addition, the infoldings near the basement membrane of the proximal tubule were not well organized. In several sections of the thin loop the cytoplasmic extensions from the nuclei were absent.

Because of the many problems we have experienced in delineating the ultramicroscopic pathology in the tubules, we do feel rather secure in our interpretations. I wonder whether a section of the kidney from your case was examined with the electron microscope and, if so, what was found.

Dr. Jack Metcoff, Chicago: I would be particularly interested to know whether you have had an opportunity to examine citrate or $\alpha$-ketoglutarate excretion in the urine. It seems obvious that these intermediates should be altered in this syndrome, given the enzymatic defect you described. I wonder if infusion of pyruvate and measurement of the amount of $\alpha$-ketoglutarate excreted might not provide a very nice test system for lack of this specific enzyme.

Dr. Robert E. Cooke, Baltimore: Could you tell us what the state of his potassium metabolism was at the time of these biopsies?

Dr. William M. Wallace, Cleveland: I am happy to see that Dr. Yaffe was helped to lay to rest the idea that renal tubular acidosis is a clinical equivalent of pharmacologic carbonic anhydrase inhibition. When the normal person receives a drug inhibiting carbonic anhydrase activity there is only a transient alkalinization of the urine. Blood pH remains low, but the ability of the kidney to acidify the urine quickly returns. Presumably the increased hydrogen ion gradient serves to offset the inhibition of the enzyme kinetics.

Dr. Philip Calcagno, Buffalo: I have a question which concerns the interpretation of the data presented on the slide showing ammonia production following an acid load. I am accustomed to thinking of ammonia production in terms of "fold" increase. For example, the premature infants in our study were able to excrete a 10-fold increase in ammonia production following an acid load of calcium chloride. These values obtained in premature infants compared well with values obtained from adults. I should like to know whether or not you related your data in a like manner, and how is this explained with the glutaminase activity observed?

My second question is in reference to the measure of $CO_2$ tension in the urine before acid loading is accomplished. It is well known that bicarbonate wasting can occur at normal or even lower levels of plasma bicarbonate concentrations. This wasting can be a factor promoting renal acidosis. Was the $CO_2$ tension determined in this case?

Dr. Yaffe: Dr. McCrory's comment about the fact that most patients with this syndrome do not have as much increase in ammonia production following an acid load is certainly correct. The same applies to the renal concentrating defect noted in most adults with the disorder. I conceive of

this syndrome as being primarily an inability of the kidney to secrete free hydrogen ions. As a result there is inability to lower the pH of the urine, with secondary hypercalcuria and pyelonephritis. As the process continues, renal damage occurs and, then, ammonia production is diminished and water reabsorption is impaired. Albright's patients and most adult patients that I have seen had decreased renal function, whereas this patient had normal glomerular filtration and normal concentrating ability. The administration of ammonium chloride (100 mEq/m²) to this patient resulted in a fall in serum pH from 7.37 to 7.30 as $CO_2$ content fell from 24 to 18 mEq/L and chloride rose from 110 to 115 mEq/L. The diminished bicarbonate diuresis (compared with normal individuals) following administration of acetazolamide may be due to the decreased action of the drug during metabolic acidosis and need not imply decreased carbonic anhydrase activity (which by actual determination was normal).

Dr. Litman, I was happy to hear that you have done electron microscopy in a patient with renal tubular acidosis, although this accompanied the Fanconi syndrome. We attempted electron microscope studies in our patient, but there was some difficulty with the fixation process and the resultant sections were uninterpretable.

Citrate excretion has been reported to be low in these patients, Dr. Metcoff, and indeed ranged from 46 to 64 mg/24 hours in this patient (normal for age 100-150 mg/24 hours). Perhaps this may be due to the high urinary pH or systemic acidosis, since citrate excretion diminishes after administration of acetazolamide, presumably secondary to the pH change induced by the drug. Dr. Cooke, this patient's serum potassium was normal at the time of biopsy. Metabolic balance studies showed a markedly positive potassium balance, which was out of proportion to the assumed tissue ratio of 3 mg. of potassium to 1 gm. of nitrogen and might suggest prior body depletion of potassium. The biopsy specimen exhibited none of the histological changes associated with hypokalemia.

In reply to the last question by Dr. Calcagno, this patient's basal ammonia production was inappropriately high for the stated urine pH. Following an acid load, there was only a two- to threefold increase in ammonia production over basal values. This was sufficient to cover 60% of the chloride increment. The normal person can increase ammonia production 10-fold in response to acidosis, but basal values are not as high as this patient's. I don't have $CO_2$ tensions, but one should be able to calculate them from urinary pH and total $CO_2$ content.

### 155. Cellular Ions and Metabolites in Rats with Diarrhea and Malnutrition

TAKASHI YOSHIDA,* JORGE MARTNER,* and JACK METCOFF, Michael Reese Hospital, Chicago

Rats develop severe diarrhea when fed lactose. Lactose, 35% w/w, was substituted for an equicaloric quantity of dextrose, after suitable control periods, in 16 of 32 weanling, pair-fed Sprague-Dawley rats. Polyuria and polydipsia developed in lactose-fed rats, and food consumption decreased. Experimental and calorie-deficient malnourished controls were killed after 14 days. Muscle and liver were analyzed for concentrations of electrolytes, pyruvate, lactate, and α-ketoglutarate.

In muscle of rats with diarrhea, ($H_2O$), (Na), and (Cl) were slightly increased; (K) and (P total) were decreased; (Mg) was unchanged (parentheses indicate content per 100 gm. DFFS). Based on the extracellular position of Cl, intracellular [Na] increased somewhat and [K] was reduced; [P] decreased markedly (brackets indicate concentration). Differences in pyruvate, α-ketoglutarate, and lactate did not seem significant. When changes of intracellular ions were related to the glycolytic metabolites, however, some interesting features of rats with diarrhea and malnutrition were evident. Relative to pyruvate, K, Mg, and "apparent" inorganic P of muscle decreased significantly. Intracellular Na increased. Similar changes were noted relative to lactate. Variable changes were observed relative to α-ketoglutarate.

As expected, pyruvate occurred in greater and α-ketoglutarate in less amounts in muscle when compared to liver. Ratios of $\left(\frac{\text{pyruvate}}{\text{lactate}}\right)$ or $\left(\frac{\text{pyruvate}}{\alpha\text{-ketoglutarate}}\right)$ in muscle and liver were unaltered by diarrhea.

These data suggest that some cellular changes in ions or certain glycolytic metabolites may be observed with diarrhea in rats; however, when variations in metabolites are related to an ionic cofactor, deviations in cell structure and possible essential energy-related metabolic sequences become more apparent. The exact physiological significance of these deviations remains to be determined but undoubtedly relates to the functions of intracellular electrolytes.

### 156. Reliability of Titan Yellow Method for Determining Serum Magnesium Levels in Patients Receiving Calcium Gluconate: Studies Primarily Related to Erythroblastotic Infants Undergoing Exchange Transfusions

CONSTANTINE S. ANAST,* University of Missouri

Introduced by Robert L. Jackson

Magnesium in the presence of sodium hydroxide and polyvinyl alcohol forms a red lake with Titan

* By invitation.

yellow. This is the basis of the commonly used colorimetric method for the determination of magnesium in body fluids. Employing this method serial serum magnesium levels were determined in infants with erythroblastosis fetalis undergoing exchange transfusions. Five hundred milliliters of blood was used in the transfusion with the administration of one milliliter of 10% calcium gluconate after each one hundred milliliters of exchange. The serum magnesium in each infant studied gradually decreased to 65% of the initial value.

The same result was obtained when an exchange transfusion with calcium gluconate administration was carried out in a puppy. No changes in serum magnesium levels were observed in puppies receiving only sodium citrate-citric acid intravenously. After the intravenous administration of 5 to 10 ml. of 10% calcium gluconate to puppies the serum magnesium, as measured by the Titan yellow method, decreased to levels comparable to those observed in the infants and the puppy given exchange transfusions with calcium gluconate. Similarly, 5 ml. of intravenous 10% calcium gluconate given slowly to normal infants resulted in equally substantial decreases in serum magnesium levels as determined by the same method.

The determination of serum magnesium by precipitation as magnesium-ammonium-phosphate was employed to compare with the results obtained by the Titan yellow method. Simultaneous determinations of serum magnesium levels by both methods were done in normal infants given 5.0 ml. of intravenous 10% calcium gluconate and an erythroblastotic infant given 5.0 ml. of 10% calcium gluconate during an exchange transfusion. No change in serum magnesium was found with use of the magnesium-ammonium-phosphate precipitation method. However, with use of the Titan yellow method the apparent decrease to 65% of the initial value was again observed.

Small amounts of calcium gluconate when added to serum or solutions of known magnesium concentrations interfered with the magnesium-Titan-yellow color development with consequent false low results. Determination of the magnesium in a pure magnesium gluconate salt by Titan yellow gave results 30% to 40% lower than the actual known value. This was further exaggerated by the addition of small amounts of calcium chloride.

Calcium gluconate is frequently used in patients where it is of interest to study magnesium metabolism. Caution is urged in interpreting magnesium determinations made by the Titan yellow method in patients receiving calcium gluconate.

Studies are in progress to further elucidate this problem.



### 157. Remarks on Glucose Transport Across the Liver Cell and on Liver Hexokinase

J. W. APPELBOOM * and WILLIAM A. BRODSKY, Louisville

The free glucose content of freshly excised dog liver was about twice that of the simultaneously collected blood, i. e., 10 mM per kilogram liver vs. 5 mM per kilogram serum. This finding was checked by (1) determination of fermentable reducing substance in Nelson-Somogyi filtrates of liver, (2) by paper chromatography of filtrates with elution of the spot and with recovery of 90% of reducing activity, and (3) by qualitatively positive glucose oxidase reaction when the reagent was applied to eluate of the spot. Analysis of such data suggests that glucose as such cannot move from interstitial fluid to liver cell unless (*a*) there exists a glucose "pump" or (*b*) the concentration of glucose in perfusing blood is at least 10 mM per kilogram. Moreover, if the free glucose inside a liver cell is as high as 10 mM per kilogram of cell water, then the reaction, glucose+ATP⇌glucose-6-$PO_4$+ADT, cannot be at or near equilibrium. Such an off-equilibrium state may be due to a vanishingly low content of ATP or to the absence of hexokinase activity in parenchymal liver cells.

#### Discussion

DR. MOLLIE COMBES, Dallas, Texas: Have you attempted to measure hepatic cellular glucose before and after insulin administration? Recent work by Madison and Combes demonstrated a fall in hepatic venous glucose output in dogs receiving slow insulin infusions. Information regarding the effect of insulin on glucose concentration within the liver cell by your methods would be of considerable interest.

DR. ROBERT KLEIN, Pittsburgh: I hesitate to speak up since I am not as sophisticated as Dr. Brodsky in this field, but I worry somewhat about some of the methodology. Our best techniques are none too good and none, even including the glucose oxidase method, are entirely specific. Furthermore, there is apparently a considerable amount of oligosaccharide on the cell surface. I don't know what influence, if any, this may have in Dr. Brodsky's system, but it may well confuse the actual measurement.

DR. WILLIAM NYHAN, Baltimore: I'd like to comment on the sources of liver glucose, a question that has been raised by these data. We recently reported data which indicated that both glutamate and succinate were very rapidly converted to glucose in rat liver in vivo (*Cancer Res.* 18:385; 1203, 1958). We had the impression from these experiments that the liver might be in the

* By invitation.

business of making glucose and might convert a number of different compounds to glucose. This concept was supported by data presented about a month ago in Atlantic City by Busch, Fujiwara, and Keer, dealing with the metabolism of radioactively labeled glucose in vivo. While most tissues are very active in the conversion of glucose to other compounds, most of the glucose arriving in the liver remains as free glucose. I want to emphasize that these data represent metabolism and not flux, and that they tend generally to support the experiments reported today.

My other comment is to the possibility of intracellular sequestration of ATP, which would explain some of the data presented. Within the liver cell ATP might, in fact, be present in high concentration, but might be so localized as to be unavailable for the hexokinase reaction.

DR. SAMUEL BESSMAN, Baltimore: This is a very elegant way of approaching this problem. One thing that must be considered in the process is that the concentration of glucose in the portal vein does not equal the peripheral blood concentration during digestion. It all depends on what is going into the intestines. When the intestinal concentration of glucose is 500 or 600 mg. %, which it is during the digestion of carbohydrate meals, then the portal concentration runs to 500 or 600 mg. %, producing a gradient in the direction of the liver cell. Now the gradient goes outward in fasting in consistence with all of Dr. Brodsky's statements and all of the physiologic evidence. The fact that hexokinase activity is not represented by the overall equilibrium still doesn't indicate that hexokinase does not function in the liver. It merely indicates that hexokinase does not contribute significantly to transport under these circumstances.

DR. BRODSKY: With regard to the first question, we didn't do experiments on the dog with or without insulin. It was on the agenda but we were diverted into other fields. I leave that to the consideration of members of the group.

With regard to Dr. Klein's questions, I could summarize and say, yes, we may be wrong about the specificity of our liver glucose measurements. Perhaps fermentation, chromatographic movement, plus glucose oxidase activity all together are not sufficient evidence for specificity of the hexose. Rigorous analysis may require isolation of the glucose and checking its exchange flux against standard radioactive glucose in a pure system. We did not do this.

I don't know about polysaccharides on the cell surface. Suffice it to say that the technique of separating glucose out of the liver was shown to eliminate glycogen, several nonglucose hexoses, and hexose phosphates.

The next question, that of Dr. Nyhan, is about the source of liver glucose. I agree that many nonglucose substrates can be converted to glucose (gluconeogenesis) by liver cells. Data of Bush et al. are of interest. The fact that most tissues converted radioactive glucose to other substances rapidly is compatible with the presence of hexokinase activity in such tissues. On the other hand, radioactive glucose presented to the liver remained therein as free glucose. This is precisely what one would expect to find if there were but little or no hexokinase activity in the liver. Sequestration of ATP is an unexcluded possibility.

In response to Dr. Bessman, if the concentration of free glucose in the portal vein exceeds that of the liver cell during digestion, glucose would diffuse from blood into liver cell. However, experiments cited were designed to avoid uncontrolled conditions present during digestion. Data were from controlled perfusion experiments where concentration of portal blood sugar was kept constant at predetermined values. An interesting observation under these conditions is the effect of epinephrine. With a given level of blood glucose, epinephrine induced the well-known increase of blood sugar. If liver cell glucose were low, glucose would have to be pumped out of the cell into the blood. It would appear to be moving outward against a chemical concentration gradient until one measures the glucose content of the liver. This we did not do, but there are data by Palmer and Cori (1917, 1923) which showed that the reduction content of liver was equal to or greater than that of blood after epinephrine. We agree with Dr. Bessman's final remark that hexokinase does not contribute significantly to these circumstances.

### 158. Pickwickian Syndrome in Children

GLEN G. CAYLER,* HARRIS D. RILEY JR.,* and JAMES MAYS,* University of Oklahoma
Introduced by Alexander S. Nadas

The Pickwickian syndrome is a well-recognized clinical entity in obese adult patients, with over 15 cases having been reported since 1936. The syndrome is characterized by obesity, cyanosis, reduced vital capacity, polycythemia, and right-sided congestive heart failure. These patients do not have primary cardiac or pulmonary disease. Alveolar hypoventilation is present and is secondary to their obesity; however, the exact relationship of the obesity to the hypoventilation as well as the subsequent complication of pulmonary artery hypertension is not at all well understood.

Two children with this syndrome are to be presented and represent to our knowledge the first reported cases occurring in childhood.

Recognition of this syndrome as a cause of cyanosis is important because of the potential dangers of oxygen administration. Apnea and

* By invitation.

| | 7-Yr.-Old | | 11-Yr.-Old | |
|---|---|---|---|---|
| | 3/17/58 | 9/29/58 | 9/29/58 | 12/19/58 |
| Weight, lb. | 180 | 105 | 144 | 122 |
| Heart failure | ++++ | 0 | ++ | 0 |
| Cyanosis | ++++ | 0 | ++ | 0 |
| Somnolence | ++++ | 0 | ++ | 0 |
| Blood pressure | 136/70 | 110/70 | 120/90 | 120/90 |
| Hemoglobin, gm/100 ml. | 18 | 11.6 | 16 | 14 |
| Albuminuria | Trace | Neg. | Trace | Neg. |
| EKG | | | | |
| (a) RVH | ++++ | ++ | +++ | ++ |
| (b) P. pulmonale | ++ | 0 | ++ | 0 |
| Systemic saturation, % | | | | |
| (a) Breathing air | 80 | 93 | 86 | 94 |
| (b) Breathing 100% $O_2$ | 90 | 98 | 92 | 100 |
| Vital capacity, % of normal | 62 | 110 | 51 | 90 |
| Time vital capacity, sec. | 95 | 72 | 95 | 90 |
| Pulmonary artery pressure | — | 40/20 | 65/25 | — |
| Pulmonary wedge pressure, mean | — | 9 | 11 | — |

death are liable to follow 100% oxygen administration, since respirations in these patients are often dependent on the stimulatory effect of arterial hypoxemia on the carotid and aortic chemoreceptors.

Clinical recognition is also important because (1) the changes associated with the Pickwickian syndrome are completely reversible after weight reduction and (2) it is a preventable illness secondary to exogenous obesity.

Clinical, laboratory, and cardiopulmonary function studies in two boys before and after weight reduction are presented in the tabulation above.

## Discussion

DR. CHARLES COOK, Boston: It is very interesting to see the Pickwickian syndrome finally described in two children.

One further complication of obesity and the Pickwickian syndrome in general deserves mention. We have seen (together with Dr. E. D. Robin) one child and several adults with this syndrome. The child that we saw was a girl, mentally retarded and markedly obese, and had most of the characteristics of the Pickwickian syndrome. This child, however, had symptoms and $O_2$ desaturation out of proportion to her decrease in ventilation. She died suddenly and at autopsy was found to have pulmonary vascular thrombi occluding many of the larger pulmonary arteries. This type of complication also occurred in the two adult patients and has been reported in the literature in one other adult. These patients, as they become bedridden because of their obesity, must have venous stasis. Thus, the possibility of pulmonary emboli should be kept in mind because, if it is thought of, it may be diagnosed ante mortem. In this connection the techniques described by Dr. Robin comparing arterial blood to alveolar $CO_2$ levels may be useful (Robin, E. D.; Julian, D. G.; Travis, D. M., and Crump, C. H.: A Physiologic Approach to the Diagnosis of Acute Pulmonary Embolism, *New England J. Med.* 260:586, 1959).

DR. GEORGE POLGAR, Philadelphia: I wonder whether you have measurements of oxygen saturation while the patient was asleep and while awake. We had some experience of this in adults, recording a drop in the ear oximeter reading as the patient fell asleep and a rise during awakening. But if the patient was kept awake by conversation, the oxygen saturation did not change.

DR. LORIN AINGER, Memphis, Tenn.: Pulmonary hypertension, presumably due to mild chronic hypoxia, occurs naturally in infant cattle which are born at an altitude above 7,800 ft. This disease known to veterinarians for a long time as brisquet disease, is characterized clinically as an acute cor pulmonale with right-sided congestive failure. This disease, apparently, is reversible, in its earlier stages. When these cows are brought down from the higher to the lower altitudes, spontaneous improvement may occur. The histological picture of the pulmonary vasculature of these animals is indistinguishable from that observed in primary pulmonary hypertension of humans.

This disease is being studied extensively in the laboratories of Dr. Hans H. Hecht in Salt Lake City, Utah. Serial catheterizations have been performed on these animals, as well as on unaffected cattle, to define the natural course of this disease and its relationship to chronic hypoxia.

DR. JERRY JACOBS, New York: Adults with this syndrome are said to break open the doors of the icebox when put on diets. Could you comment on the diet used and how you were so effective in getting this weight reduction?

DR. CAYLER: Dr. Cook, pulmonary artery hypertension secondary to pulmonary emboli is well known. I had not heard of the complication in patients with the Pickwickian syndrome.

Dr. Polgar, I have no data on sleeping versus waking saturations in these patients. I am aware that others have found saturation drops in the supine versus sitting postures, apparently owing to better aeration of the basal portion of the lungs in the sitting position. Dr. Ainger, I am not familiar with the studies on cows. Experimental hypoxia in the laboratory causes an abrupt increase in pulmonary artery pressure. The return to normal is also quite abrupt when the oxygen tension is restored to normal. Dr. Heck's cows apparently show a slow fall in pulmonary artery pressure similar to our patients.

Dr. Ainger, we had no particular difficulties in keeping these boys on their diets in the hospital. We started on a 1,200 calorie diet and decreased to 800 calories after one to two weeks. The first boy, as noted, did not continue his diet at home in spite of our efforts to help both him and his family to understand the problem.

### 159. In Vitro Red Blood Cell Uptake of $I^{131}$-L-Triiodothyronine as a Measurement of Thyroid Function in Children

JOHN F. CRIGLER JR., JUDITH HERTZ,* and MILTON W. HAMOLSKY,* Harvard

Six hundred thirty determinations of the in vitro red blood cell uptake of $I^{131}$-L-triiodothyronine (RBC uptake) as described by Hamolsky et al. (*J. Clin. Endocrinol.* 19:103, 1959) have been carried out over the past two and one-half years in infants and children with and without thyroid dysfunction. An analysis of the data on euthyroid persons with no demonstrable thyroid abnormality and on patients with untreated hypo- and hyperthyroidism is shown in the Table below.

Although experience in untreated thyroid disorders to date is limited, the results, which are similar to those reported in adults, indicate that this simple and rapid test possesses a diagnostic accuracy comparable to other technically more difficult standard methods. In addition, it permits the assessment of thyroid function where other standard methods are not applicable because of iodine (PBI) determinations were obtained during goitrous hypothyroidism where elevated protein-bound iodine apparently results from defects in hormone synthesis.

One hundred sixty-six RBC uptake determinations with simultaneously measured protein-bound iodine (PBI) determinations were obtained during the course of treating 50 hypothyroid patients with desiccated thyroid (USP), L-thyroxine, or L-triiodothyronine. In patients receiving desiccated thyroid and L-thyroxine, the RBC uptake proved as satisfactory as the PBI in following the therapeutic course. In patients receiving L-triiodothyronine, the PBI seldom exceeded 2$\mu$g. % (10% >3.5$\mu$g. in 62 determinations). In a like manner, the RBC uptake proved unsatisfactory as an objective measure of thyroid function in these patients, although 50% (30 of 62 determinations) obtained during therapy were within normal limits. Low RBC uptake values occurred at doses of L-triiodothyronine from 50$\mu$g-187.5$\mu$g. per day, amounts restoring the patients clinically to normal or increased metabolic states.

RBC uptake measurements proved as reliable as the PBI in following the therapeutic course of hyperthyroid patients receiving antithyroid drugs.

The possible significance of this test as a measure of thyroid hormone-binding to plasma proteins and tissue and its role in the study of patients with suspected thyroid disorders will be discussed.

* By invitation.

| Clinical Status | No. Determinations | Range of Values |
|---|---|---|
| Euthyroid | 80 | 10.7%-17.0% |
| Hypothyroid | 20 | 6.0%-9.8% |
| Hyperthyroid | 8 | 15.8%-27.0% |

### 160. Blood Levels of the Palmitate Ester of Chloramphenicol in Infants and Children

ALFRED DORN * and MARGARET H. D. SMITH, New York University

The blood serum levels of the esters of chloramphenicol have varied widely in the experience of different investigators. That one factor affecting such levels is the particle size of the preparation was recently reported by Glaszko and his coworkers. Among other important factors which influence these levels is the age of the subject. Data have been obtained on about 75 subjects. They show that newborn and premature infants maintain a steady level of chloramphenicol in the blood for several days after administration of a single dose of the drug. Among older infants and children there appears to be a constant pattern of inactivation and excretion, influenced, however, by simultaneous administration of certain other drugs and apparently by the presence of pathologic processes which influence liver function. Chloramphenicol curves will be presented following varying dosages of drug in patients of different ages.

### 161. Clinical Observations on the Cardiopulmonary Status of Infants with Hyaline Membrane Disease

J. E. DROBRAUGH,* A. J. RUDOLPH,* P. A. M. AULD,* R. B. CHERRY,* and C. A. SMITH, Harvard

The possibility of circulatory disturbances in the hyaline membrane syndrome has led to cardiac catheterization studies of 22 newborn infants with respiratory distress in the Boston Lying-in Hospital by Dr. A. M. Rudolph. The details of this work are being reported elsewhere. Among these is evidence of a widely patent ductus arteriosus in those infants with severe respiratory distress.

This group of infants has afforded us an opportunity to review the clinical, roentgenological, and electrocardiographic findings both before and after cardiac catheterization in an attempt to determine if there was any evidence of cardiopulmonary disturbance which might have been apparent without cardiac catheterization.

During the period of time that the cardiac catheterization study has been in progress, these clinical evaluations have been made in 135 infants. This group consisted of 12 full-term infants, 48 premature infants, and 75 infants of diabetic mothers.

The following table indicates the distribution of studies in the 63 infants with respiratory distress:

| | | |
|---|---|---|
| Infants evaluated by cardiac catheterization | 22 | |
| Infants evaluated by x-ray | 53 | (20 had cardiac catheterization) |
| Infants evaluated by electrocardiogram | 39 | (16 had cardiac catheterization) |

This study attempts to answer two questions:

1. Were the infants with the widely patent ductus arteriosus a special group not representative of the usual clinical picture of the hyaline membrane syndrome?

2. Do infants with respiratory distress and proven ductus arteriosus have clinically recognizable evidence of this particular circulatory abnormality?

## Discussion

### Of Papers by Drs. Rudolph et al. (111) and Drorbaugh et al. (161)

Dr. Bessie L. Lendrum, Syracuse, N.Y.: The catheterization data presented by the authors provide invaluable clues for uncovering the pathophysiologic processes in pulmonary hyaline membrane disease. Any postulated mechanism must explain the low systemic and pulmonary artery pressures as well as the presence of a large left-to-right shunt through the ductus arteriosus—which was not found in the nondistressed catheterized infants. A persistent patent ductus arteriosus is an unlikely primary cause since this would lead to pulmonary arterial hypertension, inasmuch as the added shunt flow should increase the normally high pulmonary arterial pressures found at this age. It is more likely that the ductus is kept open by the relatively moderate arterial desaturation found in these infants, if one may apply the studies of Dawes on fetal lambs suggesting that high arterial saturations (around 90%) act as a stimulus to contract the ductus.

The possibility that left and right ventricular failure causes the systemic and pulmonary hypotension is not compatible with the low atrial pressures found. It would be of interest to know, however, whether the authors tried digitalization of these infants, since some investigators report clinical improvement with its use.

Hypovolemia could explain the shock-like picture found. One would anticipate low pressures throughout the entire cardiovascular system, as was found, if hypovolemia were present. The congested lung fields ordinarily found on x-ray and postmortem examination are not incompatible with this concept, since redistribution of available circulating blood from the systemic to the pulmonary circulation does apparently occur through the ductus arteriosus. I wonder if the authors have measurements of the circulating blood volume in at least some of the distressed infants studied.

As the authors suggest, the shock-like picture of these infants could very well be attributed to lack of vasomotor tone because of autonomic nervous system dysfunction. Also, inadequate release of norepinephrine into the circulation may be postulated. One would anticipate beneficial results from the administration of norepinephrine as has been reported in one infant by R. J. K. Brown.

Finally, one must recall that cardiac catheterization studies give us information about one point in time. Thus, the hypotensive state may represent the end stage of a disease process which earlier had different characteristics. Dr. Neligan's data presented in an earlier paper at these meetings suggest that at least the systemic hypotension precedes the clinical symptoms, so that the catheterization data may be representative of the pathophysiologic state initiating pulmonary hyaline membrane disease.

Dr. Forrest Adams, Los Angeles: This very interesting work is going to give many of us a lot of material for thought for the next few months, possibly years.

I have several questions. One: Were there any differences in the anesthetic agents used or in gas mixtures given to the infants at the time that the catheterizations were performed?

The second question concerns the arterial oxygen saturation in the various groups. You presented only the data on your arterial oxygen saturation in the severely distressed infants. Was there any arterial desaturation in the less severely ill patient?

The third question: Do you have any serial blood pressure determination in the severely distressed babies? In other words, were they in shock right from the very moment of birth or did this develop over a period of hours?

Naturally, I would like to know how you correlate these data that you have just presented with the information that some of us have obtained indicating that there is a *significant* left-to-right and right-to-left shunt in normal newborn infants for the first several days?

Dr. Charles D. Cook, Boston: I would like to ask if the authors or any of the pathologists here know of any relationship between the respiratory distress syndrome in newborns and true persistent patent ductus arteriosus. Such a relationship if it exists would help in the interpretation of those findings of a temporary left-to-right shunt.

DR. SYDNEY SEGAL, Vancouver, B. C., Canada: In connection with Dr. Rudolph's oxygen saturation data, were these infants receiving added oxygen inhalation during the time of blood sampling?

Secondly, since Dr. Drorbaugh has linked cesarean section with the etiology of this disease, would he care to say whether he feels this operative procedure in itself may be responsible or whether there is still a possibility, as some have claimed, that some maternal or obstetrical condition for which the section was performed may be the principal etiological factor?

In connection with the proportion of infants with this disease who had required resuscitation, had a standard set of criteria served as a basis for the decision to attempt resuscitation?

Dr. Drorbaugh's reduction of a great mass of data to simple bar graphs has been a great achievement, but I believe that one important aspect of this disease has been sacrificed. This is the description of the direction and magnitude of change of clinical signs. The cyanosis and retraction on the charts should be viewed as prerequisites for the diagnosis of the syndrome and should not be interpreted as observations made on patients with this disease. However, it would be instructive to hear how the magnitude of these signs changes with this disease, to differentiate between the hyaline membrane syndrome, the aspiration syndrome, and pneumonia acquired in utero. My impression is that only in the hyaline membrane syndrome are these signs absent or minimally present at birth, increasing in severity within the first few hours of life. Silverman's retraction score would be a useful means of describing the progression of signs. In describing the respiratory rate in this disease, the bar graph provides less information than we would get from a graph showing the direction and magnitude of change of respiratory rate, as Miller has shown. I wonder if Dr. Drorbaugh would like to comment on these points.

DR. WILLIAM A. BLANC, New York: I would like to ask the author if the circumference of the ductus was measured in the cases dying with hyaline membranes and compared with the ductus circumference in cases dying with other conditions. We presented some data on these anatomical correlations at a previous meeting of the Society (*A.M.A. J. Dis. Child.* 94:533, 1957). Since then one of my former collaborators, Dr. Nodot, has done further work on the subject in Paris and reported our work in his thesis (Paris, 1958). It turns out that there is a significant correlation (at the 5% level) between the presence of pulmonary hyaline membranes and anatomical data suggesting excessive flow in the pulmonary circulation. These results certainly need confirmation, and a larger series is being studied. The data point to the fact that pulmonary overflow is probably one of the factors involved in the production of the membranes.

DR. ROBERT USHER, Montreal: I think it attests to the skill of the operators that infants who are very ill were able to be catheterized without an increase in their danger as shown by mortality rate comparison between the catheterized and noncatheterized infants. I think this points to the ability to study these infants clinically during the distress period without endangering their lives.

The questions I would like to ask Dr. Rudolph are whether serial catheterizations were ever done or whether he noticed differences in the age in which the baby was catheterized especially in relation to whether the right-to-left shunts were within the first 12 to 24 hours. In 8 out of 30 premature infants surviving the respiratory distress syndrome I have heard typical loud ductus murmurs after the third day of life, which persisted from one to four days. I would like to have the author's comments on whether these findings could be interpreted to mean that the ductus remains open longer with distress than in normals.

DR. MOSES GROSSMAN, San Francisco: I would like to ask Dr. Drorbaugh whether any comparison was made in this series between early and late clamping of the umbilical cord at the time of delivery.

DR. MILDRED STAHLMAN, Nashville, Tenn.: I wonder whether this is left ventricular failure or generalized decreased vasomotor tone in the pulmonary artery. I believe that the answer might be found if the oxygen uptake measurements were made at the same time as the pressure and A-V oxygen contents, which would allow one to calculate flows and resistances directly and decide whether or not there is a decrease in flow in the systemic circuit with a large left-to-right shunt accounting for low systemic pressure or whether or not there is a decrease in vasomotor tone. I would like to ask if oxygen uptake measurements were made so one could calculate the exact flows rather than just relative flows?

DR. DRORBAUGH: With regard to the question of digitalis, we had this in mind and we have used digitalis but not in any controlled way. We were interested that the evidence Dr. Rudolph obtained did not indicate classical signs of left ventricular failure such as elevation of left auricular pressure and we haven't at the present time pursued digitalization as a method of treatment in hyaline membrane disease.

In response to Dr. Adams' question on anesthetic agents and premedication, these factors were investigated but in contrast the factors of gestational age, cesarean section, and resuscitation in the delivery room there were no differences from group to group. The premedication experience of the sick

babies and the babies without signs of respiratory distress were essentially the same.

In response to Dr. Cook's question, I do not know of any babies known to have patent ductus arteriosus diagnosed at older age who were also known to have had hyaline membrane disease diagnosed in the immediate newborn period.

Dr. Segal's Question one: We merely wanted to record the fact of delivery (whether pelvic or cesarean section) and keep this factor in mind, not trying to draw any conclusions as to whether this factor predisposed to hyaline membrane disease.

In response to the resuscitation question, the criteria for resuscitation were the observers' own. There was no standardization by any of the recent criteria that have been set up for determining whether or not resuscitation should be given. In fact, the babies with respiratory distress were not babies who characteristically had "low Apgar scores." The resuscitation that was given in the great majority of cases was given because, after initial onset of breathing, the heart rate dropped and cyanosis persisted.

In reply to the question about timing of observations and the progression of signs, we feel that this is a very important part of the whole study. We tried to orient our clinical observations and catheterization procedures toward the early postnatal period. Our original aim was to do our studies on babies within three hours of birth. We weren't able to work this out, but in the sick babies, 90% of our observations and studies were done within the first six hours of birth. Our idea here was to study the infant at the time his disease was developing in order to study the pathogenesis of the disease.

We have not measured the size of the ductus at postmortem examination. We have felt that this measurement is not relevant to the size of the ductus in life.

In answer to Dr. Grossman's question about early and late clamping of the cord, we have no information on this point.

Dr. Rudolph: In reply to the first discusser's questions regarding the congested lung field, I feel that this is merely an indication of the fact that there is fluid in the lungs and I don't really think that in itself it can be of much help in evaluating the various etiological factors.

We certainly appreciate that the blood volume is a very important factor in determining pressure in the vascular system and we do not have measurement on levels of blood volume, but certainly the available information suggests that the blood volume in these infants is higher rather than lower, although we certainly have no direct measurements on this.

We have not measured arterial oxygen tension but with arterial saturations of 93% I would hardly feel that these tensions were significantly reduced.

In reply to Dr. Adams' questions, I would first like to mention that we do not use any medication of any sort in these patients. These studies were done without any anesthesia or medication of any type.

The arterial saturations in the infants who did not have severe respiratory distress, in all except one, were well up in the 90% to 95% range. One infant who had a low saturation was an infant whom we felt had atelectasis in one lung and took some time to recover, after a difficult period of resuscitation.

I am afraid I cannot correlate for you the exact infants in whom Dr. Nelligan made serial blood pressure determinations. I think that in a few instances, he studied the blood pressure in infants who were later subject to catheterization and found low blood pressure, but I'm not sure of that point.

Now, in reply to the question regarding the ductus, there is no good information for the presence of an open ductus in these babies immediately after birth. I would like to stress particularly the point that I made, and that is that the ductus is not anatomically closed and I'm sure the various influences may allow this duct to open again. I think that the fact that these babies were well saturated may explain one of the differences as seen in Hultgren's studies and some of the studies of Dr. Adams.

One other problem we found is that it was almost impossible in most of these infants to keep the catheter out of the ductus. The catheter just took its course straight through the ductus as soon as it entered the pulmonary artery and we found in some of our earlier studies that we weren't quite sure whether we were sampling pulmonary arterial blood unless we got the catheter out into a right or left pulmonary artery. As you well know, this is no simple achievement in these babies. However, we used an extremely soft catheter and we did manage to get the catheter into the right or left branch of the pulmonary artery. I think that possibly some of the observations of the very large shunt through the ductus could be explained by the fact that the sampling was right at the ductus almost in the aorta where it was felt that the catheter was in the pulmonary artery.

In reply to Dr. Segal's question regarding the question of percentage oxygen administration, we did use oxygen administration on all these infants as we demonstrated and described in the slide.

The question regarding the dynamics of the ductus and the physics of the ductus, I'm not

quite sure that I quite understood. I think one really gets into all sorts of difficulties trying to evaluate ductus size in a pulsatile system without knowing the length of the ductus, phasic differential pressure, and phasic flow through the ductus.

In reply to Dr. Usher's question, we did not in any instance repeat the catheterization studies in these babies. We have only one single study, and again I'd like to stress this, that this is only one observation, during the whole period of the illness. We do not have any repeat studies on these infants.

In reply to Dr. Stahlman's question about oxygen uptake, we have not measured oxygen uptake. We now have a method for measuring oxygen uptake but we have no data to report on this.

I'm sure I have not answered many questions but I would like to thank you for the discussion.

### 162. A Simple Partition Method for the Measurement of Free and Conjugated Bilirubin in Serum

WALTER R. EBERLEIN,
Children's Hospital of Philadelphia

A simple partition method has been devised for the measurement of free bilirubin, bilirubin monoglucuronide, and bilirubin diglucuronide. Serum, 0.1 or 0.2 ml., is dissolved in a standardized buffer and extracted with ethyl acetate/butanol in stoppered test-tubes. The mixtures are shaken and centrifuged, and the separate layers are read in the Beckman DU spectrophotometer. Separations of the free from conjugated forms of bilirubin by this method were checked by column chromatography. The sum of the three components measured in this way agrees satisfactorily with the total bilirubin content of sera determined by the diazo reaction. The sum of the monoglucuronide and diglucuronide usually agrees with the amount of "direct bilirubin" measured by the diazo reagent; however, in some sera only part of the conjugated bilirubin is measured by the diazo reagent, which leads to a spurious elevation of the "indirect bilirubin" content.

The partition method has been applied to the study of jaundiced sera and compared to indices of erythrocyte destruction and liver function in the same patients. In erythroblastosis almost all the serum bilirubin exists in the free form; small amounts of the monoglucuronide are found, but the diglucuronide is absent. In two infants, however, after exchange transfusion, large amounts of the monoglucuronide were found, which had in part been measured as "indirect bilirubin" by the diazo reaction. In biliary atresia of infancy or in older subjects with extrahepatic obstruction conjugated bilirubin accounts for 60%-90% of the total bilirubin, and the diglucuronide predominates. With developing hepatocellular damage, as well as in cases of hepatitis, the monoglucuronide level rises and becomes predominant.

The partition method is simple, rapidly performed, and superior to column chromatography in the avoidance of protein precipitation. It permits both analysis of "direct bilirubin" and a more accurate assay of unconjugated bilirubin in small amounts of serum. The method has proved useful in the differential diagnosis of jaundice. Its value in following the course of hepatitis and the need for retransfusion in hemolytic disease of the newborn is under study and will be reported.

### 163. The Nephrotic Syndrome Induced by Penicillamine Therapy

FRANCIS X. FELLERS* and
NASROLLAH T. SHAHIDI,* Harvard
Introduced by Randolph K. Byers

Penicillamine (dimethyl cysteine) has been used in the treatment of hepatolenticular degeneration to increase copper excretion. This report describes the appearance of a syndrome characterized by anasarca, proteinuria, elevated serum cholesterol, and decreased serum albumin in a 16-year-old boy under treatment with this drug. A diagnosis of hepatolenticular degeneration had been made two years previously with the appropriate laboratory studies and biopsy samples.

Penicillamine therapy, 1 gm. daily, resulted in significant copper excretion in the urine which, however, decreased within two months. Dosage was raised to 2 gm. daily and continued until the abrupt onset of generalized swelling of the face noted nine months later. The urinary and serum analyses confirmed the diagnosis of the nephrotic syndrome. Renal biopsy was obtained, and the alterations will be presented.

Fourteen days after the withdrawal of the Penicillamine massive diuresis resulted in a weight loss of 36 lb. and reversal of the serum and urinary abnormalities.

Reinstitution of the Penicillamine, 1 gm. daily, resulted in the appearance of proteinuria on the 7th day and edema on the 14th day, at which time the drug was discontinued. Fourteen days after omission of the Penicillamine, diuresis again occurred with disappearance of the protein from the urine. Subsequent urinalysis has shown no protein.

It would appear that the nephrotic syndrome of this patient was related to the Penicillamine treatment. Therefore, dimethyl cysteine may be included in the list of etiological agents of the nephrotic syndrome, such as certain heavy metals, the analeptic 2-4-dione drugs, and aminonucleoside.

* By invitation.

### 164. Study of Basic Data on Pulmonary Function in Children

JUAN FILLER,* PATRICIA FLEMING,* and MARILYN PORTER,* New York University
Introduced by Margaret H. D. Smith

We selected a group of 128 children, of both sexes, admitted to the clinic and wards of Bellevue Hospital for reasons other than cardiopulmonary diseases. Their age ranged from 5 to 12 years, and in all of them height, weight, body surface area, and chest circumference were determined.

The following measurements were carried out:

1. Vital capacity and subdivisions by spirometry and functional residual capacity by McMichael's helium dilution technique, with the child in supine position.

2. Maximum breathing capacity in a spirometer of 9 liters capacity, with very low resistance; the child was in the upright position and constantly encouraged to breathe deep and fast for 15 to 20 seconds.

All lung volumes were corrected for body temperature and barometric pressure and saturated with water vapor.

Significant correlation was found between the lung volumes and height and the maximum breathing capacity and body surface area.

Gases in arterial blood were measured in more than 40 children approximately in the same age range as those on whom lung volumes were obtained. Blood was taken from the brachial artery by means of a Cournand needle which remained in place for at least 30 minutes. When the so-called "steady state" was achieved, the blood was drawn and simultaneously the minute ventilation was measured.

It was possible to exercise most of these children by stepping up and down from a stool 20 cm. high, 30 times a minute, timed by a metronome; a second sample of arterial blood was drawn immediately after the exercise and the ventilation measured.

In both samples of arterial blood, at rest and exercise, the following determinations were made: oxygen content, oxygen capacity, and from the relation of both, the oxygen saturation, the $CO_2$ content, pH and by means of nomograms the $pCO_2$.

Samples of the expired gases during the "steady state" and during the exercise were taken and analyzed for $O_2$ and $CO_2$. Consequently the oxygen uptake and carbon dioxide output were determined and corrected to standard temperature and pressure and dry.

All the preliminary data obtained have been subjected to statistical analysis.

* By invitation.

### 165. Comparison of the in Vitro Metabolism of Tetrahydrocortisone in the Fetal, Newborn, Pregnant and Nonpregnant Adult Rat Liver

ROBERT E. GREENBERG,* and LYTT I. GARDNER,
State University of New York, Syracuse

The concept that the newborn animal and human has an ineffective mechanism for the conjugation of glucuronides has been supported by numerous studies in other laboratories, using such substrates as bilirubin, o-aminophenol, acetanilide, and salicylates. Hydrocortisone, however, undergoes a sequential process of reduction of the A-ring before conjugation with glucuronic acid is possible. The study of the metabolism of steroids, then, must separate factors limiting reduction of the A-ring as contrasted to those affecting the conjugating mechanism.

Tetrahydrocortisone was incubated with rat-liver slices in Kreb's bicarbonate buffer for one hour at 38 C in an atmosphere of 95% $O_2$-5% $CO_2$. After extraction with chloroform and chromatography on a Florisil column, the amount of steroid lost from the medium was determined per 100 mg. wet liver by means of the Porter-Silber reaction. The liver of the newborn rat was found to metabolize less tetrahydrocortisone per 100 mg. tissue than that of the nonpregnant adult rat. However, there was no significant difference in the rate of conjugation of tetrahydrocortisone in the 19-day-old fetus, newborn, pregnant, or postpartum rat. No sex difference was found in adult animals. Studies done at 7:30 a. m. and 3:30 p. m. with liver slices from nonpregnant adult females indicated no diurnal variation in tetrahydrocortisone metabolism.

With the qualification that the in vivo conjugation of tetrahydrocortisone in the rat may not involve the formation of glucuronides, studies were done to evaluate the effect of bilirubin and glucuronic acid on the in vitro metabolism of tetrahydrocortisone in liver slices. No enhancement or inhibition of tetrahydrocortisone metabolism was observed in all groups studied.

These studies suggest that similar factors may be responsible for the quantitative reduction in the in vitro metabolism of tetrahydrocortisone found in fetal, newborn, and pregnant rat liver as compared to normal adult animals. It seems likely that more significant rate-limiting effects of delayed enzymatic maturation in the metabolism of hydrocortisone in the fetus and newborn will be found in the study of the highly specific enzymes involved in the initial reduction of the A-ring.

#### Discussion

DR. MELVIN GRUMBACH, New York: Last night Drs. Ducharme, Morishima, and I presented a paper at the Endocrine Section on the rate of disposal of hydrocortisone and its metabolites from

plasma in premature and newborn infants and on the ratio of free to conjugated steroids in the urine of premature infants following the administration of ACTH and various C21 steroids. The results indicated, as Dr. Greenberg has so nicely shown here, that the major defect was impaired reduction of Ring A of the steroid molecule rather than an impaired ability to conjugate the reduced metabolites of hydrocortisone with glucuronic acid, although a few infants had both defects. These subjects showed a defect in hydrocortisone metabolism similar to that observed in patients with liver disease.

I don't believe that the available evidence permits one to conclude at this time that impairment of Ring A reduction is necessarily due to a deficiency of enzymes. Recently Fouts and Adamson have demonstrated that extracts obtained from the liver of newborn rabbits inhibited the metabolism of certain substrates in vitro by adult rabbit liver preparations. Dr. Greenberg, have you investigated the possibility of an inhibitor of the enzyme system which brings about reduction of Ring A in your fetal and neonatal liver preparations?

DR. ROBERT A. ULSTROM, Minneapolis: Studies such as Dr. Greenberg has recorded must take into account the possibility of other enzymatic reactions occurring simultaneously with the reaction one is actually testing for. In the final analysis, several such actions may become additive in such a way that the results will be altered. In this case, I wonder if the presence of $\beta$-glucuronidase was specifically noted which could split the glucuronide steroid complex as soon as it was formed. I also wonder whether Dr. Greenberg attempted to determine the specificity of his methodology by actually recovering the steroid glucuronide or the glucuronic acid split from this complex to be sure that no other type of steroid complexes were split in this system.

We have studied this problem in newborn infants by changing the concentration of substrate and have found that this type of change could be interpreted as influencing enzymatic activity. During the first week of life, newborn infants excrete approximately 20% of Porter-Silber chromagens in the urine as free material. If one administers 10 mg. per kilogram of cortisol, however, the rate of excretion of steroid glucuronide rises from a mean of approximately 5$\mu$g. per hour to 40$\mu$g. per hour. This suggests that a step preceding the union of steroid and glucuronic acid may, in part, be responsible for the failure to conjugate an average of 20% of the steroidal material excreted. We have also found that this free material is almost entirely 6-$\beta$-hydroxycortisol and very little unmetabolized cortisol is excreted. This implies that some of the failure to form conjugated material is due to a pathway of metabolism which results in an unconjugatable metabolite of cortisol, namely, 6-$\beta$-hydroxycortisol. We recovered only 4%-8% of the injected cortisol as Porter-Silber chromagens; even using labeled cortisol, we find that only 15% of radioactivity can be extracted with organic solvents. There must be many other compounds as yet undetected which are of this nature.

The comparison of steroid to bilirubin conjugation is an obvious one. The type of conjugation with glucuronic acid, however, is quite different in the steroid glucuronide as compared with the bilirubin glucuronide complex. One wonders, therefore, whether the specificity of glucuronyl transferase is of such low degree that it may partake in one reaction which results in an ether linkage and another in which the result is an ester linkage. It seems likely that two similar, but not identical, enzymes may be involved; therefore, information obtained regarding the glucuronide conjugation of the steroid may not always be transferable to mechanisms of bilirubin conjugation.

### 166. Studies of Free Norepinephrine and Epinephrine Excretion in the Newborn

ROBERT E. GREENBERG* and LYTT I. GARDNER,
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The adrenal medulla of the adult human contains predominantly epinephrine, with norepinephrine comprising 17% to 20% of the total medullary amine content. In recent years, however, it has been shown that the adrenal medulla of the fetus and newborn contains almost entirely norepinephrine, with a gradual change to the adult proportions of both amines by the age of 1 to 2 years. The physiological significance of and factors responsible for this maturation of catecholamine content in the adrenal medulla are unknown. A differentiated secretion of epinephrine and norepinephrine from the adrenal medulla occurs in adults, at least, depending on the functional requirements of the organism. Accordingly, epinephrine is released in response to insulin, for example, whereas mainly norepinephrine is released in response to hypotension. The presence, then, of catecholamines almost entirely in the form of norepinephrine in the medullary and extramedullary chromaffin tissue must be of considerable significance to the fetus and newborn.

The normal 24-hour urinary excretion of free norepinephrine and epinephrine in the adult varies between 6$\mu$g. and 80$\mu$g. per day, with norepinephrine accounting for 60% to 90% of the total. The values of norepinephrine are a resultant of secretion from chromaffin tissue and the autonomic nervous system, whereas the excretion of epinephrine is a reflection of the adrenal medulla only. In other laboratories the recovery of free cate-

* By invitation.

cholamines after parenteral administration to adults has been found to comprise only 1% to 4% of the amount injected.

The experimental data of our study are as follows: the 24-hour urinary excretion of free norepinephrine and epinephrine was determined in 1- to 5-day-old infants. The chemical method of estimation involves conversion of both amines to their respective iodochromes with iodine and subsequent formation of their respective trihydroxyindoles with strong base. The amount of each amines is determined by differential oxidation at pH 6 and 3.5. The fluorimetric readings were obtained by the use of an Aminco-Bowman spectrophotofluorometer. The combined catecholamine content of newborn urine ranged from 0.6μg. to 1.9μg. per 24 hours, with norepinephrine comprising 75% to 100% of the total.

Subcutaneous injection of 50μg. to 100μg. of epinephrine in divided doses over a 1-hour period resulted in the recovery in 24-hour urine collections of less than 5% of the injected amount as free epinephrine. While the role of reduced glomerular filtration rate in the newborn cannot as yet be evaluated with respect to these results, this would suggest that the full-term newborn manifests no deficiency in the metabolism of injected epinephrine as compared to the adult.

### 167. The Metabolism of Hydrocortisone and Its Metabolites in Premature and Newborn Infants: Evidence for Defective Degradation and Impaired Hydrolysis of Steroid Ester

M. M. GRUMBACH, J. R. DUCHARME,* and A. MORISHIMA,* Columbia

During studies designed to determine the excretion of "free" and "conjugated" 17-hydroxycorticosteroids (17-OHCS) in premature infants, we noted an increased ratio of free to β-glucuronidase-liberated 17-OHCS in a high percentage of these subjects. The altered ratio was demonstrated more readily following the rise in 17-OHCS excretion produced by corticotropin (ACTH), suggesting a relative insufficiency in the enzymatic mechanisms involved in the catabolism of 17-OHCS.

To clarify the nature of this abnormality in hydrocortisone metabolism, the rate of clearance from plasma of hydrocortisone (F) and its hemisuccinate ester, and of tetrahydrocortisone (THE) and tetrahydrohydrocortisone (THF) were studied in premature and newborn infants. The excretion of free and conjugated 17-OHCS in the urine also was determined, and the steroids, partially characterized. Following the intravenous injection of steroid, small samples of venous blood were withdrawn at intervals from 5 to 420 minutes by a method developed in this laboratory. The rate of disappearance of plasma 17-OHCS was calculated from a semilogarithmic plot of the data.

Seven full-term infants less than 24 hours of age were infused with 7-10 mg. per kilogram of body weight of hydrocortisone hemisuccinate. The curve of disappearance of plasma hydrocortisone showed two major components. In five out of six subjects in whom determinations were made in the first three hours after steroid administration, a gradual rise in the concentration of 17-OHCS was observed, followed, after an interval, by an apparently logarithmic fall. The gradual rise in the level of plasma 17-OHCS suggests a defect in the hydrolysis of the hydrocortisone ester. The mean half-times as calculated from the later portion of the disappearance curves showing an exponential rate of fall was 220 minutes (range 102-340 minutes), a rate of clearance about one-half that obtained in subjects over one month of age.

The half-time in five infants 24-72 hours of age was slightly less (mean, 193; range, 99-315 minutes); an early delay in the rate of fall of plasma 17-OHCS was also noted. The rate of clearance of plasma hydrocortisone tended to vary directly with the dose of steroid administered.

In five infants less than one day of age, the rate of removal of plasma hydrocortisone was observed after infusion of 4 mg. of hydrocortisone alcohol per kilogram. The mean half-time was 126 minutes (range, 93-187). A delay in the early part of the disappearance curve similar to that observed with hydocortisone hemisuccinate was not found. In three subjects 3 to 9 days of age, the mean half-time of plasma hydrocortisone was 101 minutes (range, 72-116); however, in two, the disappearance curve showed a delay in the first two hours followed by a sharp and constant rate of fall. Similar results were obtained in six premature infants 2-4 weeks of age. The removal rate of infused THE and THF was studied in three subjects less than one week of age; in one, the clearance rate was delayed.

The relationship of these observations to such factors as blood pH, serum bilirubin levels, corticosteroid-binding protein, and the possible effects of other hormones will be discussed.

The data suggest that in some premature and newborn infants there is a reduced rate of reduction of the $\Delta^4$-3-ketone in Ring A; in some instances, both reduction of Ring A and the formation of glucuronide conjugates of the reduced steroid metabolites of F are impaired. Bongiovanni et al. recently have described a similar abnormality in Ring A reduction in the newborn infant, but the half-life of hydrocortisone (administered as the hemisuccinate) reported by these workers is considerably greater (mean, 977 minutes) than that which we have found.

* By invitation.

Reduction of Ring A of the hydrocortisone molecule and its subsequent conjugation with glucuronic acid, which occurs principally in the liver, have been shown to be catalyzed by enzymatic mechanisms. The defect in hydrocortisone metabolism in the premature and newborn infant is in many ways comparable to that found in cirrhosis of the liver. In addition, we have obtained evidence of a new enzymatic defect, namely, an impaired ability of the immature infant to hydrolyze hemisuccinate esters.

The results of this study again point out the variable maturity of enzymatic mechanisms in a group of premature and newborn infants and the importance of considering this factor when administering steroids, or other substances, such as chloramphenicol (Chloromycetin) and streptomycin, which are metabolized in a similar manner.

### 168. Influence of Skin Testing on the Serologic Diagnosis of Tuberculosis and Histoplasmosis

DOUGLAS C. HEINER,* University of Arkansas

Introduced by Theodore C. Panos

Within the past year, reports have appeared in the literature suggesting the utility of precipitin reactions in agar gel in making the diagnoses of tuberculosis and histoplasmosis. One of the reports indicated that histoplasmin skin tests frequently stimulate the production of high titers of circulating antibodies in sensitized persons. The present study was undertaken to determine more fully the effects of tuberculin and histoplasmin skin tests on serum antibodies with use of agar diffusion techniques.

Nineteen healthy medical students and house officers were given an intradermal injection of 1:1,000 Wyeth old tuberculin. Those having a positive reaction with greater than 1.5 cm. induration received no subsequent injections. Those with 0.5 to 1.5 cm. induration were given two to four repeat skin tests weekly in order to attain a strong antigenic stimulus. Negative skin reactors, including all who had less than 0.5 cm. induration, were given an average of five weekly injections of a 1:100 dilution of the same tuberculin. Control serum samples obtained from each person prior to skin testing were negative. Precipitin bands were ultimately produced by the sera of seven persons. One serum produced two precipitin bands; all others, only a single band. Each precipitin band is presumed to represent a minimum of one antigen-antibody system. In no instance was a high titer of antibodies produced as determined by dilution studies.

Circulating antibodies appearing after skin tests are shown in the tabulation.

| Skin-Test Material | No. Persons | No. with Pos. Skin Tests | No. Pos. Precipitin Tests: Control | No. Pos. Precipitin Tests: 2-4 Wk. |
|---|---|---|---|---|
| Wyeth old tuberculin (Lot 58802) | 19 | 16* | 0 | 7† |
| Parke-Davis histoplasmin (Lot unknown) | 32 | 10 | 0 | 9‡ |
| Lilly histoplasmin (Lot AX22570-B) | 19 | 11 | 0 | 13§ |

* Seven of sixteen were negative to intradermal injections of 1:1,000 old tuberculin but positive to 1:100.

† Five of the seven sera were from persons with a positive test to 1:1,000 old tuberculin, one, from a person positive only to 1:000, and one from a person showing erythema but no induration on four successive skin tests.

‡ All positive skin reactors produced demonstrable circulating antibodies except one.

§ Two developed a low titer of circulating antibody in response to repeated negative skin tests.

Fifty-one medical students and house officers were given intradermal histoplasmin injections, thirty-two receiving Parke-Davis and nineteen receiving Lilly skin test materials. Repeated tests were given to weak or negative reactors. Control sera, obtained before skin testing, were negative in each instance. Twenty-three persons had serial serum specimens drawn during the first week after the initial skin test to determine how soon circulating antibodies appeared; all had weekly serum samples for five or more weeks. Circulating antibodies were detected in the sera of three highly sensitized persons as early as five days after a positive skin test. Antibodies were commonly demonstrable 10 days after the initial injection and were present in high titer within 2-3 weeks. Three of five positive skin reactors studied 10 months following intradermal injection still maintained a significant titer. Most sera demonstrated two precipitin bands, but several produced three distinct bands; a few produced only one. All persons produced *m* antibody when any response was detected to either histoplasmin skin test material. No person produced *h* antibody in response to Parke-Davis histoplasmin, but two produced a high titer in response to Lilly histoplasmin. These findings are believed to be related to a general high degree of antigenicity of *m* substance, as well as a marked relative increase in *h* antigen concentration in the Lilly histoplasmin.

It is concluded that histoplasmin and tuberculin skin tests may in themselves cause the production of circulating antibodies, particularly in persons with strongly positive intradermal reactions, thus requiring caution in the interpretation of serologic reports following skin tests. Antibodies due to skin testing have not been detected earlier than five days after an initial intradermal injection. Therefore, it is suggested that serum specimens

* By invitation.

for antibody studies should be drawn within four days of the time of a skin test, preferably at the time of the injection or when the reaction is read at 48 hours. The only justifiable exceptions would seem to be those instances where the antibody response to a specific skin testing antigen has been carefully determined by prior study of a large number of sensitized persons. A helpful first step would be standardization of the antigenic composition of all commercial skin test materials.

### 169. Congenital Nephrosis

KWANG W. KO * and FRANCIS X. FELLERS,*
Harvard
Introduced by Randolph K. Byers

The nephrotic syndrome occurring in the neonatal period has been termed congenital nephrosis, with the inference that the disease is different from that occurring in other age groups. A male infant was found to have 4+ albuminuria at the age of 4 weeks while under treatment for an ear infection. Appropriate laboratory studies confirmed the diagnosis of the nephrotic syndrome. Moderate generalized edema was present.

Observation on the metabolic service included six balance periods of three to six days each before and during a course of corticotropin (ACTH). The balance data as shown are very similar to that from studies carried out in older nephrotic children. An unusually positive nitrogen balance was associated with a nitrogen-to-potassium ratio of 1:8. The calcium balance was positive throughout which would be in keeping with the normal radiological appearance of the bones.

The corticotropin decreased the positive nitrogen balance as well as the percentage absorption of phosphorus, magnesium, and calcium from the bowel. The diuresis was associated with a return toward the precorticotropin balance values but with a significant negative sodium and chloride balance.

Hypomagnesemia was a prominent feature during the diuresis of this infant. Because no significant increase in urinary magnesium occurred in this patient although there was a decrease in magnesium absorption from the intestinal tract, the red cell magnesium was determined. Serial analyses failed to show any change in magnesium expressed as microequivalents per gram of hemoglobin either in this infant or in four other children during corticotropin-induced diuresis. Other cellular shifts are not precluded.

At the age of 8 months the infant still showed activity of the nephrotic syndrome. These data would suggest that there is no significant difference in the biochemical features studied of the different childhood types of the nephrotic syndrome.

The balance data are summarized in the tabulation.

| Period | Control (% Intake Absorbed) | Corticotropin | Diuresis |
|---|---|---|---|
| Nitrogen, mg/kg/day | 156 | 66 | 197 |
| Potassium, mM/kg/day | 1.2 | 0.7 | 1.6 |
| Phosphorus, mM/kg/day | 0.9 (63) | 0.4 (49) | 1.2 (44) |
| Magnesium, mM/kg/day | 0.2 (30) | 0.1 (19) | 0.5 (26) |
| Calcium, mM/kg/day | 1.1 (35) | 0.5 (12) | 1.0 (38) |

* By invitation.

### 170. Apparatus for Establishment of Separate Extracorporeal Fetal and Maternal Circulations in the Human Placenta

KERMIT E. KRANTZ * and THEODORE C. PANOS,
University of Arkansas

Previous studies of extracorporeal placental circulation have been limited to perfusion of the fetal vascular system. The development of a duel closed circulation apparatus which will maintain the placenta for long periods would immeasurably facilitate experimental study of placental functions. Such an apparatus has been devised which appears, from preliminary investigations, to satisfy the criteria of double, intact, functioning circulations.

The DeWall Constant Dual Perfusion Pump is employed. The umbilical arteries and vein are cannulated, with the vein draining fetal blood from the placenta into a reservoir. Blood leaving this reservoir is passed through one pump, regulating pressure and flow into the umbilical arteries. This forms a complete fetal circuit. Because oxygenation normally occurs via maternal-fetal transfer, there is no oxygenator in this circuit.

A cylindrical plastic container (2.5 cm. deep and 20 cm. in diameter), simulating the uterine bed, has been constructed which allows for regulated arterial flow through the intervillous space and return to a venous system which enters a modified Mark III type oxygenator. The placenta is fitted into the container in such a manner that the fetal surface and umbilical cord are free. Through perforations in the base of the container and the basal plate of the placenta, multiple jets of oxygenated adult blood matched with maternal blood are delivered into the intervillous space. Return blood enters the oxygenator, whence it passes to the other pump for pressure and flow regulation and transmission to the intervillous space.

To date, various dyes and particulate substances have been introduced into both systems in order to demonstrate separate and intact circulations. Microscopic sections of the placenta, taken after

prolonged (several hours) operation of the apparatus, have shown no morphologic damage to or distortion of placental structure, specifically the villi. Particulate matter (e. g., India ink) did not pass into the other circulation following introduction into either, while a variety of dyes and substances in solution did transfer across the barrier at different rates. Dissection of the placenta following instillation of dye into the maternal circuit indicated that not less than 80% of the intervillous space was successfully perfused; following instillation of dye into the fetal circuit, there was apparently complete distribution.

### Discussion

DR. SYDNEY SEGAL, Vancouver, B.C., Canada: Mr. Chairman, it is most delightful to have this organ system come within the scope of pediatrics, and to have it presented to us by a professor of obstetrics.

Several possible applications of this tool come immediately to mind, but to consider one or two questions about the method: Has any thought been given to the inclusion of an oxygen user on the fetal side of the circulation?

Secondly, since pumping would be continued for a prolonged period, does hemolysis become a problem? Have any high levels of free hemoglobin in the plasma been observed and has some method been found to reduce the hemolytic process?

DR. A. LEONARD LUHBY, New York: The apparatus developed by Drs. Krantz and Panos is an important contribution for the experimental study of placental function. We have been studying the mechanism of the placental transfer of vitamin $B_{12}$ from mother to fetus by administering tracer doses of radioactive $B_{12}$ to the pregnant woman before birth and obtaining a sample of cord blood to measure transfer. With the latter technique there are significant limitations in the experimental plan with regard to the size of the radioactive tracer that can be used because of safety considerations to mother and infant as well as the inability to obtain frequent samples of the fetal blood. The apparatus designed by these authors will permit such experimental procedures as were not previously possible, and will immeasurably add to the advancement of knowledge of placental function. I did not hear mention of the size of the maternal and fetal pool of blood used in your apparatus. From the illustration, it looks large. If so, this may be a "reagent" of considerable expense.

DR. ARTHUR MCELFRESH, Philadelphia: In how many placentas have you been able to find the complete separation between the maternal and fetal sides in respect to the passage of particulate matter? I ask since we have previously felt that in a number of mothers red cells from the infant reached the mother at the time of parturition, and that this was responsible for a fair share of isoimmunization to blood group antigens.

DR. JOSEPH DANCIS, New York: Would you describe how you enter the intervillous space in order to establish the circulation on the maternal side of the placenta? Do you perforate the decidual plate?

DR. ROBERT E. COOKE, Baltimore: Would you tell us the length of time it takes between the delivery of the placenta and the fixation in the apparatus, because I would think that these membranes might change their characteristics fairly rapidly in an anoxic environment.

DR. KRANTZ: I want to thank Dr. Panos personally, as he has worked devotedly with me on this project. His ideas have been excellent.

Firstly, we have not employed an oxygen user in the fetal system nor have we determined the placental needs as yet. Hemolysis in the system has occurred. It is minimal. We have a greater problem with clotting and we have recently been able to control it through introduction of fibrinolysin. In fact, this has aided the perfusion of the intervillous space, reducing the clotting from the high levels of thromboplastin in the placenta which promotes immediate clotting.

As to fetal blood in the maternal system—hence a break—in the placentas studied thus far we have demonstrated in most of them no evidence of a break. The amount of the blood and plasma present in the maternal system is approximately 1,500 cc., with another 500 cc. of fetal blood in the fetal system. I would like to point out that there is about 250 cc. of fetal blood in the placenta at birth and 200 cc. of maternal blood in the intervillous space.

As to adequate perfusion of the intervillous space, this is achieved through careful puncturing of the basal plate by the apparatus. At present we are using plastic needles. The basal plate is rather thin and can be very easily punctured, not necessarily interrupting the villi.

The length of time from parturition to establishment of the placenta in the apparatus has been as little as five minutes.

It can be noted that Chesley in 1940 while doing cultures on the sections of cotyledons in the Lindbergh-Carrel apparatus was able to keep the tissue alive as long as 90 days. He found that the tissue would survive even up to one hour after parturition. Apparently time is not as significant as one might think in so far as we know at this time Of course, this is a preliminary report. We hope that as we begin to study the various aspects of the transfer and metabolism many questions will be answered concerning maternal-fetal and fetal-maternal relationships.

Thank you for the privilege of being here. I have enjoyed it very much.

### 171. Vectorcardiogram in the Differential Diagnosis of Atrial Septal Defect

JEROME LIEBMAN,* ELLIOT YOUNG, and ALEXANDER S. NADES, Harvard

The commonest types of atrial septal defects are (*a*) the high so-called ostium secundum defect frequently associated with pulmonary venous anomalies and (*b*) the low, ostium primum lesion almost invariably accompanied by clefts in the mitral and tricuspid valves and occasionally associated with a high ventricular septal defect. In order to encompass the entire spectrum of possible variations involving defects low in the atrial septum, high in the ventricular septum, and in one or both atrial atrioventricular valves, the entire complex will be discussed under the embryological term of endocardial cushion defect.

The importance of differentiating endocardial cushion defects from ostium secundum lesions is twofold: (*a*) The prognosis is considerably worse in the former than the latter and thus surgery is more urgent, and (*b*) whereas a number of closed surgical techniques and hypothermia may be successfully applied for correction of ostium secundum lesions, only open-heart surgery by means of a pump oxygenator is capable of solving the complex deformities of endocardial cushion defects.

The purpose of this presentation is to delineate the vectorcardiographic differences between the two lesions, since the electrocardiogram—the tool hitherto most frequently used in differentiation—does not invariably clarify the diagnosis. As a background to this work, brief reference will be made to our normal vectorcardiograms in children. Finally, an interpretation of the vectorgraphic patterns will be attempted in the light of hemodynamic data.

The Grishman cube technique was used throughout the study.

Thirty-two definite ostium secundum defects and twenty-five proven cushion defects were studied. In addition, six unusual cases will be presented: four secundum atrial septal defects with clefts in the A-V valves and two ventricular defects with tricuspid clefts.

The characteristic vectorcardiographic patterns were as follows: (*a*) The frontal plane angle in every one of the secundum defects—including those associated with mitral clefts—was positive (0 to +180). Contrariwise, all but three of the cushion defect patients had a negative axis (0 to −180). The axes of the three exceptional patients were 0, +10, and +30, respectively. (*b*) The direction of inscription in the body of the frontal plane was counterclockwise in all but one endocushion defect, the one exception being a patient with marked pulmonary hypertension. Conversely, 26 of the 32 secundum defects showed clockwise inscription in the frontal plane. The remaining six had only narrow loops.

It is interesting to note that the features cited above were independent of the hemodynamic patterns, the negative axis being present despite evidence of marked right ventricular hypertrophy. Thus, it is suggested that the left-axis deviation may be the result of a specific pattern of conduction. Right ventricular hypertrophy, on the other hand, manifests itself in both lesions by more anterior and rightward direction of all forces, as well as changes in the direction of the inscription in the sagittal and horizontal planes.

### 172. Physiologic Effects of Infant Swaddling

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State University of New York, Syracuse

Swaddling in the care of infants is a practice common to many cultures. There has been considerable speculation concerning its effects on the infant. The current study was undertaken to observe the effect of swaddling on physiologic processes.

Ten newborn infants were repeatedly tested (average of three and one-half different sessions) in the postprandial state in a manner previously employed in this laboratory. The stimulus was a jet of oxygen of 2½ lb. pressure and five seconds' duration via a plastic cone taped in place above the umbilicus. During each session the infant was stimulated in semirandom fashion a total of 30 times while swaddled and 30 times while allowed motor freedom. Respiratory rate, movement, cardiotachometer measurements of heart rate and skin temperature were continuously recorded on a polygraph in a constant temperature laboratory.

As in our previous studies, a highly significant inverse relationship appeared between prestimulus heart rate level and stimulus induced change in heart rate. Thus, at lower prestimulus levels large changes occurred; at high prestimulus levels little change or a slowing of rate occurred.

The heart rate during *each* of the seconds during the 5 seconds of stimulation and 10 seconds following stimulation was measured for statistical analysis. Tests of statistical significance were obtained where possible, by means of an analysis of variance. This analytical technique provides a relatively complete picture of the heart-rate response of the organism and allows for more extensive comparisons between and within individual persons than have been previously reported.

Swaddling consistently produced a state characterized by lower average prestimulus heart rates, less crying, more sleep, and generally decreased respiratory rate. However, the effect of swaddling upon response to stimulation was not as consistent, since

* By invitation.

eight individual babies characteristically demonstrated a decrease, while others an increase or no change in heart rate reactivity.

The significance of these findings for an understanding of the clinical implications of the cultural practice of swaddling will be discussed.

### 173. Evidence for a Bond Between Ribonucleic Acid and Glycogen Induced by Cortisone in Rat Liver

CHARLES U. LOWE, University of Buffalo

The accumulation of ribonucleic acid (RNA) in hepatocyte cytoplasm during anabolism satisfies current theory concerning the role of polynucleotides in protein synthesis. Less well understood is the role of glycogen in this cycle. Protein synthesis is poor in glycogen-depleted livers and also decreased in the glycogen-rich liver of the cortisone treated rat. Study of the relation between RNA and glycogen in rat liver after cortisone administration revealed an apparent chemical bond between these substances which may explain the decrease in protein synthesis occurring under the influence of this hormone.

Rats received 25 mg. of cortisone acetate by intramuscular injection for five days and were fasted for 24 hours before killing (Group I). Control rats were either fed (Group II) or fasted for 24 hours (Group III) before killing. Glycogen content of liver in milligrams per gram in each group was as follows: Group I, 90-100; Group II, 50-70; Group III, 2-5. Sodium ribonucleate was isolated by conventional extraction methods, and attempts were made to precipitate RNA independantly of the accompanying glycogen. In Group I, virtually all RNA precipitated with glycogen; in Group II only a portion of the RNA precipitated with glycogen; in Group III, coprecipitation was negligible. When the sodium nucleate was depolymerized by heating in 1 N NaOH, RNA-free glycogen was obtained. Under these circumstances, however, up to 10% of the ultraviolet (UV) absorbing material present in the original sample appeared with the glycogen. Extensive dialysis failed to remove significant UV absorbing material from the glycogen. When the dialyzed glycogen was reprecipitated from dilute acid, up to 90% of the remaining UV-absorbing material appeared in the supernatant (Fraction AS). Paper chromatography and ultraviolet-absorption spectroscopy revealed the presence of adenine, guanine, cytosine, and uracil in this fraction. The glycogen was still associated with some UV-absorbing material, which could only be removed by hydrolysis in hot acid. The UV-absorbing material under these circumstances (Fraction GP) proved to be only adenine and guanine. The fed and cortisone-treated animals were similar with respect to composition of AS and GP. The fasted animals had negligible amounts of GP. Within two hours of administration of glucagon to cortisone-treated animals, glycogen in the liver fell nearly to fasting levels and values for GP were very low.

These data indicate that a bond may exist between glycogen and RNA via two purines, adenine and guanine. The role of the purines and pyrimidines removed by dilute acid is not clear. In the cortisone-treated animal all cytoplasmic RNA acts as if bound to glycogen. If this binding is through phosphate, the poor staining of RNA with basic dyes in such livers would be explained. Whereas in the liver of normal fed animals RNA and glycogen are also bound, there is still a considerable amount of unbound RNA. If RNA is indeed bound to glycogen, as these data would indicate, it is readily conceivable that such bound RNA would not serve as an effective template for protein synthesis. It is furthermore attractive to speculate that an adenine or guanine-carbohydrate molecule may be the source of energy for protein synthesis.

#### Discussion

DR. JOHN M. CRAIG, Boston: Dr. Lowe has made me very happy because he has given an explanation for an observation which I made several years ago. In investigating the use of the Hale mucopolysaccharide stain, I found that rabbit liver when filled with glycogen gave a very positive reaction which disappeared promptly along with the periodic acid-Schiff stain after exposure to malt diastase. Of course, in the usual crude preparations of diastase there are probably other proteolytic enzymes. However, if there is, as Dr. Lowe said, a close combination of some of the acid groups of the ribonucleic acids with the glycogen, one may well have a sufficient number of acid groups to give a positive Hale reaction.

In contrast to the glycogen-filled liver, in the liver which contains little glycogen the cytoplasmic ribonucleoprotein is usually clumped in large masses and will give a positive metachromatic stain with the thiazine dyes, the metachromatic staining being due to a polymerization of the dye bound to the repeating closely spaced acidic groups. In a liver filled with glycogen, one gets a less strongly active metachromasia, and this change may be due to the dispersion of the acid groups over the cell on the glycogen, and no longer by close apposition would they be able to induce the metachromatic reaction.

### 174. An Attempt to Prevent Hyperbilirubinemia of Prematurity Using N-Acetyl-p-Aminophenol

JEROLD F. LUCEY * and TIMOTHY J. DRISCOLL JR.,*
Vermont

Introduced by R. J. McKay Jr.

There exists a need for a better method than exchange transfusion in handling hyperbilirubinemia of prematurity.

We believe that a means of *preventing* hyperbilirubinemia would have definite advantages.

Our hypothesis was that the enzyme system concerned with bilirubin excretion might be an adaptive one which begins to function adequately only when presented with a high level of serum bilirubin on the fourth to sixth day of life. If this enzyme system is nonspecific it might be possible to administer early a substance excreted by the same system and thus activate it on the first day of life. N-acetyl-*p*-aminophenol (Tylenol) was chosen as a nontoxic substance predominantly excreted by conjugation with glucuronic acid.

A total group of 60 premature infants were assigned on a random, double-blind basis to one of two groups.

Thirty infants received 60 mg. Tylenol drops q. 4 h. from 12 hours to 36 hours of age. The control group received a placebo.

The groups were comparable with respect to birth weights, hemoglobin levels, number of infants with respiratory distress, mortality rate and potential incompatibility of ABO blood groups.

Serum bilirubin levels were done on the second, fourth, and sixth days of life. There were no significant differences found in mean bilirubin levels between the groups nor in the number of falls in bilirubin levels occurring between the fourth and sixth days of life in either group.

We conclude that N-acetyl-*p*-aminophenol as tested in this study had no effect on hyperbilirubinemia. This material also did not have any detectable influence on mortality rate, body temperatures, or respiratory rates of this group of premature infants.

* By invitation.

### 175. Problems of Biological Variation: Serum Glutamic-Oxalacetic Transaminase (S-GOT) Activity from Birth to Old Age

A. J. SCHNEIDER,* Syracuse
and Communicable Disease Center, Atlanta

Introduced by Julius B. Richmond

Any practical solution to the problem of the biological variability of a particular measurement requires (1) that the "norms" can be established from a reasonably small number of measurements on any given person and (2) that the established "norms" be of predictive value for assessing as yet unstudied persons. Repeated measurements of S-GOT activity in 180 healthy infants and children and in 427 healthy adults will be used to assess (*a*) variation between selected groups of persons, particularly by age, (*b*) variation between persons within a specified group, and (*c*) variation of a person's activity about his own mean value. A method combining the technique of analysis of variance and quality control statistics will be presented as a partial answer to the common assertion that a measure of a person's variations is desirable but not practical in the above sense.

Preliminary analysis of the data showed that S-GOT activity varied significantly with age, but not with sex, race, or degree of maturity. While there was a statistically significant sex difference of about 2 Karmen units after age 20 years, this difference is not of practical significance. The age trends are summarized in the accompanying Table.

The rapid changes in S-GOT activity in early infancy are even more striking in individual infants. Values have ranged from 30 to 565 Karmen units in the same infant and at varying periods of time. No infant, however, had activities consistently in excess of twice the median value for age. These enormous variations made meaningful estimates of within-individual variation impossible.

The suggested upper limits of normal represent an attempt to set figures which include about 95%-98% of the healthy population. The remaining 2%-5% of the population, however, may exceed

| Age | No. Observations | S-GOT Activity, Karmen Units | | | | |
|---|---|---|---|---|---|---|
| | | Median | Mean | Range | S. D.* | Upper Limit |
| 0-24 hr. | 74 | 93.1 | 112 | 35.9-420 | -- | 190 |
| 5-10 days | 62 | 53.5 | 61.0 | 25.6-370 | -- | 110 |
| 25-31 days | 30 | 35.2 | 39.8 | 23.9-101 | -- | 70 |
| 92-121 days | 13 | 54.5 | 75.4 | 37.4-210 | -- | 110 |
| 2 yr. | 16 | 40.0 | 43.2 | 33.1-56.0 | 5.9 | 60-65 |
| 9 yr. | 20 | 36.0 | 36.6 | 26.2-50.3 | 5.9 | 55 |
| 20-70 yr.† | 418 | 24.2 | 25.5 | 10.6-77.1 | 6.8 | 80 |

* Variation between persons within age groups, standard deviation.

† Males and females combined. Values from nine persons ranging from 22-115 Karmen units excluded. If these values included, mean is 26.2, S. D. 9.5.

the suggested limits by factors of 2-5 for as yet unknown reasons. Such deviations result from the skewness of the distributions of values at all ages.

From multiple observations on persons over 2 years of age, varying in number from 2 to 5, and in time interval from one week to six months, analysis of variance yielded a standard deviation for variation of a person about his own mean value of 4.0 Karmen units. From this value it can be predicted that the range of S-GOT activities observed in a healthy person on the basis of two or more determinations made within a six-month period will seldom exceed 22 Karmen units. The theoretical incidence of persons exceeding this limit is 0.2%, while the observed incidence was 2%-3% of presumably healthy persons, irrespective of age, sex, or mean activity.

The method for quantitatively assessing within- or intraindividual variability employed here should be generally applicable for many kinds of attributes whenever ill-defined changes in the attribute with time are not too rapid. The range limit, so defined, provides an accessory criterion of normality and may be especially useful in deciding "how much" change is significant.

### 176. Serum Transaminase Variations in Infectious Hepatitis

A. J. SCHNEIDER* and JAMES MOSLEY,*
Communicable Disease Center, Atlanta

Introduced by Julius B. Richmond

Asymptomatic household contacts of cases of infectious hepatitis were compared with children in families with no known exposure to hepatitis with respect to the incidence of persons showing abnormalities in S-GOT activity. Persons with a range of S-GOT activity exceeding 22 Karmen units in a series of 2 to 5 determinations over a one- to three-month period were considered to have abnormal S-GOT activity. It was assumed that if a significant difference in incidence of abnormals between such groups could be shown, then the test would be useful as a method of detecting subclinical cases of infectious hepatitis.

Families with hepatitis and control families were studied during the course of a community-wide epidemic of infectious hepatitis in Ely, Nev. In Atlanta, Ga., families with hepatitis occurring as sporadic cases were studied, while a control group was selected from families in which one or more children had recently been seen in a local clinic for illnesses other than hepatitis. The following results were obtained:

| Family Group | | Normal S-GOT, No. | Abnormal S-GOT No. | Abnormal S-GOT % |
|---|---|---|---|---|
| Ely, Nev.: | Hepatitis | 7 | 6 | 46 |
| | No illness | 16 | 1 | 6 |
| Atlanta, Ga.: | Hepatitis | 11 | 7 | 39 |
| | Other illness | 37 | 3 | 8 |

Results from both areas under vastly different epidemiologic conditions were very comparable and were statistically significant ($P<0.025$). It was concluded that a person with (*a*) a range of S-GOT variation in excess of 22 Karmen units, (*b*) exposure to a clinically recognized case of infectious hepatitis, and (*c*) no other condition known to cause S-GOT abnormality may be considered to have infectious hepatitis himself, whether symptomatic or not.

Two additional family groups, one from Ely, Nev., the other from Atlanta, Ga., studied during a focal outbreak of hepatitis were composed of members who received immune globulin prophylactically within three weeks after onset of a case of infectious hepatitis. Similar studies of S-GOT variation were made in the children of these families with the following results:

| Family Group | | Normal S-GOT, No. | Abnormal S-GOT No. | Abnormal S-GOT % |
|---|---|---|---|---|
| Ely, Nev.: | With globulin | 8 | 4 | 33 |
| | Without globulin | 7 | 6 | 46 |
| Atlanta, Ga.: | With globulin | 24 | 1 | 4 |
| | Without globulin | 11 | 7 | 39 |

In this instance, the difference between the Atlanta groups was highly significant ($P<0.025$), while no significant differences was demonstrable between the Ely groups. These findings suggest but do not establish that immune globulin given under appropriate but as yet unknown circumstances will prevent not only clinical disease but also the S-GOT abnormalities associated with infectious hepatitis.

Finally, observations were obtained which supported the following conclusions:

1. Ordinarily S-GOT activity becomes abnormal within a few days prior to onset of symptoms of infectious hepatitis.

2. The S-GOT abnormality may recede within a few weeks after onset of symptoms but may persist or recur for periods of at least several months in some 40% of patients.

3. S-GOT abnormalities in completely asymptomatic household contacts of cases of hepatitis were comparable in magnitude and duration with those seen in clinically recognized cases.

4. No correlation between magnitude and duration of S-GOT abnormality and degree of clinical illness was observed.

* By invitation.

### 177. The in Vivo Occurrence of Bilirubin Crystals

TERESA VIETTI,* Wayne
Introduced by Wolf W. Zuelzer

The mechanism whereby hyperbilirubinemia produces kernicterus is unknown. One of the postulated mechanisms is injury of the cells by indirect bilirubin.

The new observations and photomicrographs which will be shown reveal crystals of indirect bilirubin within the white blood cells of the peripheral blood, bone marrow, and cerebrospinal fluid obtained during life in 11 infants with jaundice.

The presence of crystalline bilirubin within the cytoplasm of cells may be indicative of the degree of injury caused by the hyperbilirubinemia of that particular patient and, therefore, may be a measure of the clinical severity of the illness, or possibly these intracellular crystals indicate interference with the metabolism of the cells and could be correlated with the clinical and pathological findings of kernicterus.

The technique of detecting such crystals in materials taken during life, the clinical data of the individual patients, and the postmortem findings in the nine patients so studied will be given, as well as a discussion of the possible significance of these observations.

### 178. Observations on Methods for Detecting Primaquine-Sensitive Erythrocytes

WILLIAM H. ZINKHAM, Johns Hopkins

Several biochemical abnormalities have been described in erythrocytes from primaquine-sensitive persons: a deficiency of reduced glutathione (GSH), an accelerated fall of GSH after incubation of blood with acetylphenylhydrazine (GSH stability test), a marked reduction of glucose-6-phosphate dehydrogenase (G-6-P. D.), and an increase in aldolase and glutathione reductase activity. Two in vitro techniques are currently available to detect persons with primaquine-sensitive red cells: the GSH stability test and assay for G-6-P. D. in in hemolysates. During the course of family studies to further characterize the sex-linked transmission of these abnormalities, a small percent of Negro females ("intermediate females") were observed in which there was no correlation between the GSH stability test and G-6-P. D. values. Some demonstrated moderately abnormal GSH stability tests with normal enzyme activity, while in others the GSH stability test was normal and enzyme levels minimally reduced. Three Negro women who should have been heterozygotes on the basis of family studies had normal GSH and enzyme studies.

Quantitation of G-6-P. D. activity in the red cell was previously measured by determining the rate of reduction of triphosphopyridine nucleotide (TPN) at 340 mμ with glucose-6-phosphate as substrate, readings being made between 5 and 15 minutes after initiating Reaction 1 by the addition of TPN. One unit of enzyme was defined as that amount which reduces 1.0μM of TPN per minute. Reactions 1, 2, and 3† are the first three steps of the hexosemonophosphate shunt, and all of the enzymes for this metabolic sequence are present in hemolysates. Therefore each mole of G-6-P oxidized in Reaction 1 can eventually form 2 M of TPNH under optimal conditions, so that any assay for G-6-P. D. using G-6-P as substrate and quantitated by rate of TPNH formation may give falsely high values. Experimental evidence to support this hypothesis has been derived by determining initial rates of TPNH formation with G-6-P as substrate Reduction of TPN during the first 5 minutes of the reaction is 15% to 30% of that observed between 5 and 15 minutes.

To separate G-6-P. D. activity (Reaction 1) from that of 6-PG. D. (Reaction 3) Glock and McLean have devised the following assay technique. Rate of TPNH formation is measured in one cuvet with 6-PG as substrate and in the other with G-6-P and 6-PG as substrates. G-6-P. D. activity is calculated by subtracting the value of the former from that of the latter (derived G-6-P. D. value). Assays using this method have been done on red cells from 29 normal white adults, 10 "intermediate" Negro females and their families, and 3 Negro female reactors and the results compared to those obtained with G-6-P as substrate. Derived G-6-P. D. values for all the intermediate Negro females except one were definitely less than those observed in normals. In the one exception the enzyme value was 121. Four intermediate Negro females who were mothers of reacting sons or daughters had normal GSH stability tests and low normal G-6-P. D. levels with G-6-P as substrate. Their derived G-6-P. D. values, however, were abnormal: 65, 80, 106, and 95 units of enzyme per 100 ml. RBC. The findings indicate that the

* By invitation.

† These reactions are:

$$\text{(1) G-6-P} + \text{TPN}^+ \xrightleftharpoons{\text{G-6-P. D.}} \text{6-phosphogluconolactone} + \text{TPNH} + \text{H}^+$$

$$\text{(2) 6-phosphogluconolactone} \xrightarrow{\text{Lactonase}} \text{6-PG (6-phosphogluconate)}$$

$$\text{(3) 6-PG} + \text{TPN}^+ \xrightleftharpoons{\text{6-PG. D. (6-phosphogluconic dehydrogenase)}} \text{Ru-5-P} + \text{CO}_2 + \text{TPNH} + \text{H}^+$$

$$\text{Sum of reactions: G-6-P} + 2\ \text{TPN}^+ \longrightarrow \text{Ru-5-P} + \text{CO}_2 + 2\ \text{TPNH} + 2\ \text{H}^+$$

| | Normal White Adults (29) | Intermediate Negro Females (10) | Reacting Negro Females (3) |
|---|---|---|---|
| G-6-P. D. (G-6-P as substrate) | 211 * (173-278) | 147 (86-176) | 32 (21-63) |
| Derived G-6-P. D. | 149 (120-201) | 70 (35-121) | 10 (6-12) |

* Units of enzyme per 100 ml. RBC.

derived G-6-P. D. method is more sensitive than other in vitro procedures for detecting persons with primaquine-sensitive erythrocytes.

### 179. The Effect of Carbonic Anhydrase on Erythrocyte Potassium Loss in Vitro

T. H. SHEPARD * and P. K. DATTA,*
University of Washington

Introduced by Dr. Robert A. Aldrich

Much indirect evidence has lead to the hypothesis that carbonic anhydrase may be related to hydrogen and potassium ion excretion from the renal tubule. The contributions from many scientific fields have resulted in a general theory that hydrogen and potassium ions compete for a common excretory mechanism, reduction of one ionic species inside the cell enhancing the excretion of the other species.

In this communication the theory has been directly investigated using human erythrocytes as a model system. Factors which control the rate of penetration of acetazolamide (Diamox) into the erythrocyte were thoroughly studied. When human erythrocytes are suspended in isotonic sucrose the potassium is rapidly depleted from the cells, as shown in the Table.

| Inhibitor | Final Conc., μg/Ml. | K, mEq/L. of RBC * | % Excess K Loss * | Carbonic Anhydrase Inhibition % |
|---|---|---|---|---|
| None | -- | 51.5 | 0 | 0 |
| Acetazolamide | 100 | 46.7 | 5.7 | 26.3 |
| Acetazolamide | 300 | 40.3 | 10.1 | 47.5 |
| Acetazolamide | 500 | 34.5 | 16.0 | 68.8 |
| Sulfanilamide | 500 | 55.5 † | −2.9 | −2.5 |
| Sulfathiazole | 500 | 55.5 † | −0.9 | 0 |

* After two hours' incubation. The percentage excess loss was calculated from the original concentration which varied slightly.
† In these experiments the potassium content of the control experiment varied from 48.8 mEq. per liter to 57.3 mEq. per liter.

The close relationship between carbonic anhydrase inhibition and excess potassium loss may support the theory that carbonic anhydrase actively is related to membrane transfer of potassium.

* By invitation.

## News and Comment

### GENERAL NEWS

**Wyeth Pediatric Fellowships Awarded to Twenty Physicians.**—The recipients of the 1959 Wyeth Laboratories pediatric residency fellowship were announced on July 13 by Dr. Philip S. Barba, past president of the American Academy of Pediatrics and chairman of the fellowship selection committee.

The pediatric program, sponsored by the Wyeth Fund for Postgraduate Medical Education, provides a grant of $4,800 to each physician, thus enabling him to spend two years of advanced study in the care and treatment of children.

Those receiving the fellowship awards include the following: Drs. Robert E. Bartholomew, Farmer City, Ill.; Robert A. Campbell, San Francisco.; E. Christine Costly, Liberal, Mo.; Neil P. Duncanson, Seattle, Wash.; Frederick W. Floyd, Gloucester, N.J.; Lowell A. Glasgow, Cincinnati; Robert P. Hardman, Waterville, Ohio; Charles W. Lewis, Murfreesboro, Tenn.; Elmer S. Lightner, Carlisle, Pa.; Lee G. Miller, Grass Valley, Calif.; Frank Mitchell Jr., Chicago.

Also, Robert L. Olson, Lindsborg, Kan.; Frank A. Oski, Philadelphia; Harry C. Powell Jr., Houston, Texas; Raymond J. Rademacher, Missoula, Mont.; Charles I. Sheaffer, Acton, Ind.; James W. Stackpole, Burlington, Vt.; Thomas F. Stallings, Smithfield, N.C.; William B. Wood, Lowell, Mass., and Ray C. Wunderlich, St. Petersburg, Fla.

The recipients, from 16 different states, are the second group to benefit from the program established last year by the Philadelphia pharmaceutical manufacturing firm.

Selection was made on the basis of interest in pediatrics, character, able performance of duties, and academic competence. The Wyeth fellowship winners may attend any hospital whose residency is accredited by the Residency Review Committee of the American Board of Pediatrics and the Council on Medical Education and Hospitals of the American Medical Association.

The fellowship committee is composed of five eminent pediatricians from different sections of the nation. Dr. Barba, the chairman, is associate dean and associate professor of preventive medicine and pediatrics at the University of Pennsylvania School of Medicine.

Other members include the following: Dr. John A. Anderson, chairman of the pediatrics department, University of Minnesota; Dr. Amos Christie, who holds a similar position with Vanderbilt University; and Dr. Hugh A. Carithers, Jacksonville, Fla., and Dr. Crawford Bost, San Francisco, both engaged in private pediatric practice.

**Heart Association Seeks a Guide for Public in Multiple Health Drives.**—The American Heart Association announced on July 24 that it would seek the appointment of "an impartial group of physicians, scientists and community leaders" to study the problems arising from the increasing number of fund appeals by health agencies. Dr. Francis L. Chamberlain, President of the Association, said the intent of the proposed study would be "to create better understanding of the primary health needs of the nation, and at the same time to provide a yardstick for adequate and intelligent support of those health causes which are of greatest concern to the greatest number of people."

Dr. Chamberlain said that approval had been given by the Association's Board of Directors to a resolution calling for the appointment of the study group. According to the resolution, the proposed committee would (1) define the major areas of chronic disease that threaten the nation's health and well-being; (2) set up standards by which potential contributors and volunteers could evaluate the health causes which seek their support.

Public confusion resulting from the increasing number of health appeals has "diverted attention," the resolution declared, "from the urgent goal of conquering the major chronic diseases which constitute the greatest threat to the health of the nation." It pointed out, "United Funds and federated campaigns have not provided satisfactory solutions to the problem of multiple health appeals. Furthermore, they are not constituted to provide the leadership and support for the research and other programs essential to the reduction of disability and death caused by the major chronic diseases."

The action by the American Heart Association's Board of Directors was taken, the resolution stated, because "the public interest urgently requires that the utmost support be given to the voluntary health agencies dedicated to the conquest of these major chronic diseases."

The full text of the resolution by the Association's Board of Directors follows:

### Resolution on Multiple Health Drives

"The increasing number of appeals for support made by health agencies has resulted in public confusion. It has diverted attention from the urgent goal of conquering the major chronic diseases which comprise the greatest threat to the health of the nation.

"United Funds and federated campaigns have not provided satisfactory solutions to the problem of multiple health appeals. Furthermore, they are not constituted to provide the leadership and support for the research and other programs essential to the reduction of disability and death caused by the major chronic diseases.

"Since the public interest urgently requires that the utmost support be given to the voluntary health agencies dedicated to the conquest of these major chronic diseases, the Board of Directors of the American Heart Association hereby resolves to seek the appointment of an impartial and qualified group of physicians, scientists and community leaders to undertake a study of the problem of multiple health appeals with the objectives of:

"1. Defining the major areas of chronic disease which present the greatest threat to our national health and welfare;

"2. Establishing criteria which will guide the public in making voluntary contributions of time, effort and funds to individual health causes."

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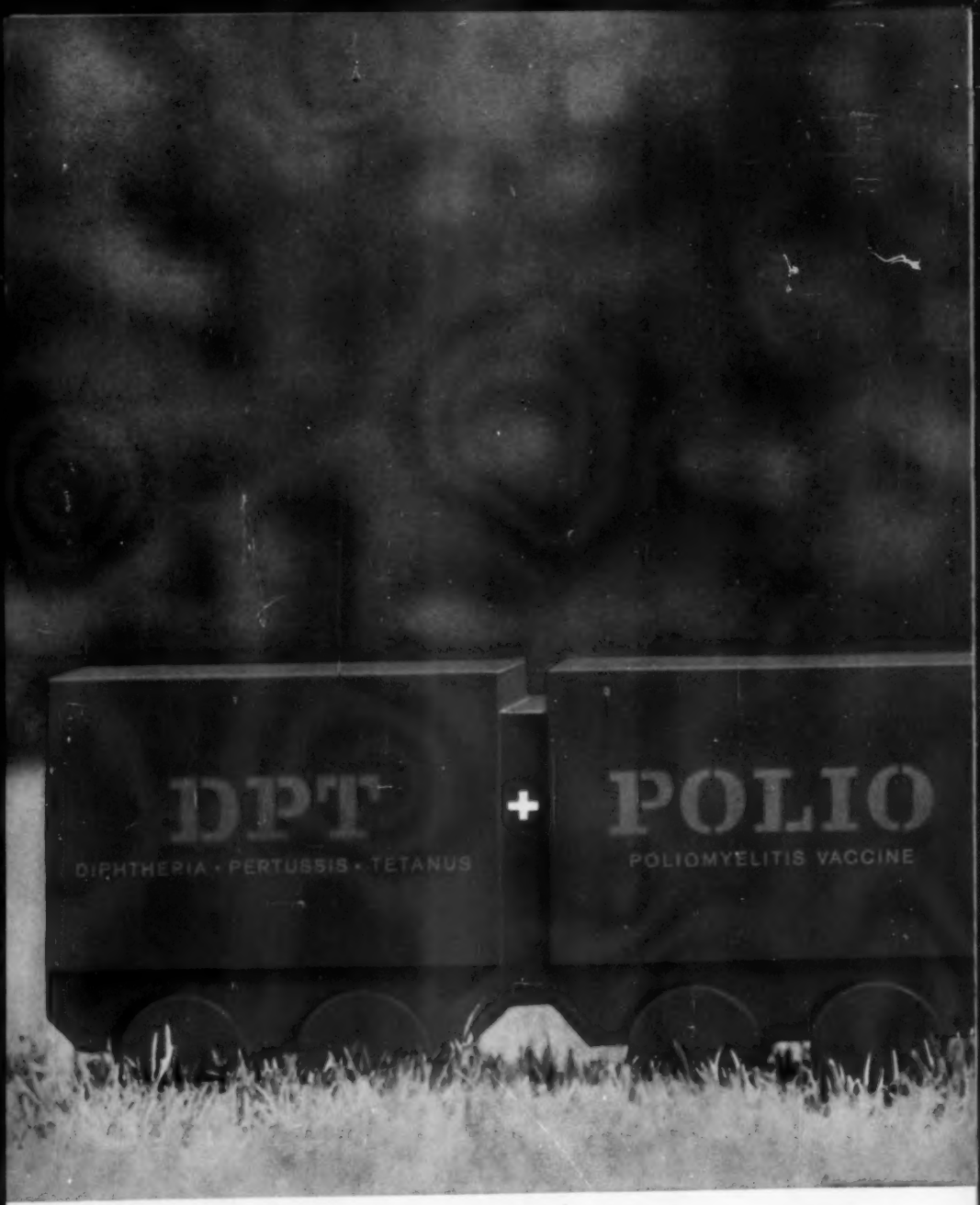


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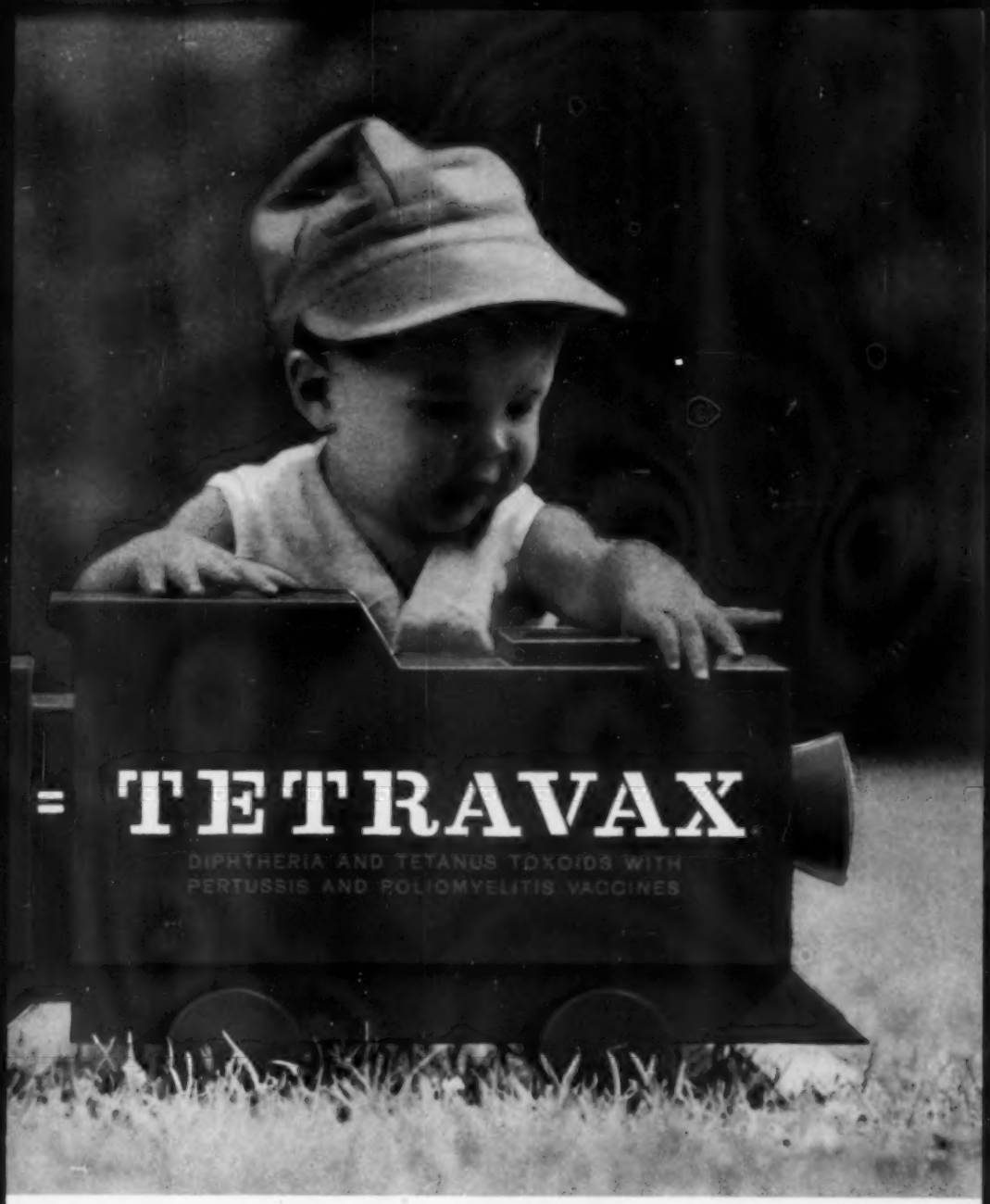


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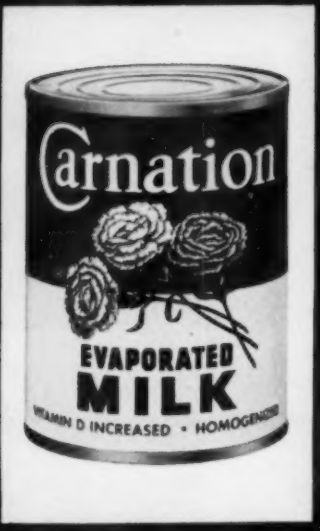

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*...and for continued, compatible, coordinated therapy*

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*oxytetracycline with glucosamine*

## CAPSULES

Continuation with oral Cosa-Terramycin every six hours will provide highly effective antibacterial serum and tissue levels for prompt infection control.

The unsurpassed record of clinical effectiveness and safety established for Terramycin is your guide to successful antibiotic therapy.

*Supply:*
*Terramycin Intramuscular Solution**
100 mg./2 cc. ampules
250 mg./2 cc. ampules

*Cosa-Terramycin Capsules*
125 mg. and 250 mg.

*Cosa-Terramycin is also available as:*
*Cosa-Terramycin Oral Suspension* — peach flavored, 125 mg./5 cc., 2 oz. bottle
*Cosa-Terramycin Pediatric Drops* — peach flavored, 5 mg./drop (100 mg./cc.), 10 cc. bottle with plastic calibrated dropper

Complete information on Terramycin Intramuscular Solution and Cosa-Terramycin oral forms is available through your Pfizer Representative or the Medical Department, Pfizer Laboratories.

*Contains 2% Xylocaine® (lidocaine), trademark of Astra Pharmaceutical Products, Inc.

*Pfizer*
*Science for the world's well-being™*

PFIZER LABORATORIES, Division, Chas. Pfizer & Co., Inc., Brooklyn 6, N. Y.

# A Laxative Modifier of Milk for CONSTIPATED CHILDREN

**Borcherdt's**
**MALT SOUP EXTRACT**
**(MALTSUPEX)**
**POWDER**

## is a food supplement and not a drug.

Nature is helped to function normally because Malt Soup Extract Powder promotes the favorable growth of aciduric bacteria in the lower tract and tends to create and maintain soft natural stools. There are no gas pains, no habit forming tendency, no dangerous impaction.

Malt Soup Extract Powder is a specially processed non-diastatic barley malt extract neutralized with potassium carbonate. It can be given over a long period of time (when necessary) with complete safety and no side effects. Children love its mild malt flavor when mixed with milk or it may be used on cereals or in fruit juices. Two tablespoonfuls (heaping) of Malt Soup Extract Powder twice a day is the usual effective dose. Two forms are available, powder and liquid, in two sizes 8 oz. and 16 oz. jars.

***We'll gladly send clinical samples of powder and (or) liquid.***

**Borcherdt Company**

217 North Wolcott Avenue,
Chicago 12, Illinois

In Canada:
Chemo Drug Co. Ltd.,
Toronto, Ontario

Borcherdt Company CP
217 N. Wolcott Ave., Chicago 12, Ill.

Gentlemen: Please send samples and literature of your Malt Soup Extract (Maltsupex).

☐ Powder ☐ Liquid

M.D.

Address _____

City _____ Zone _____ State _____

# NEW

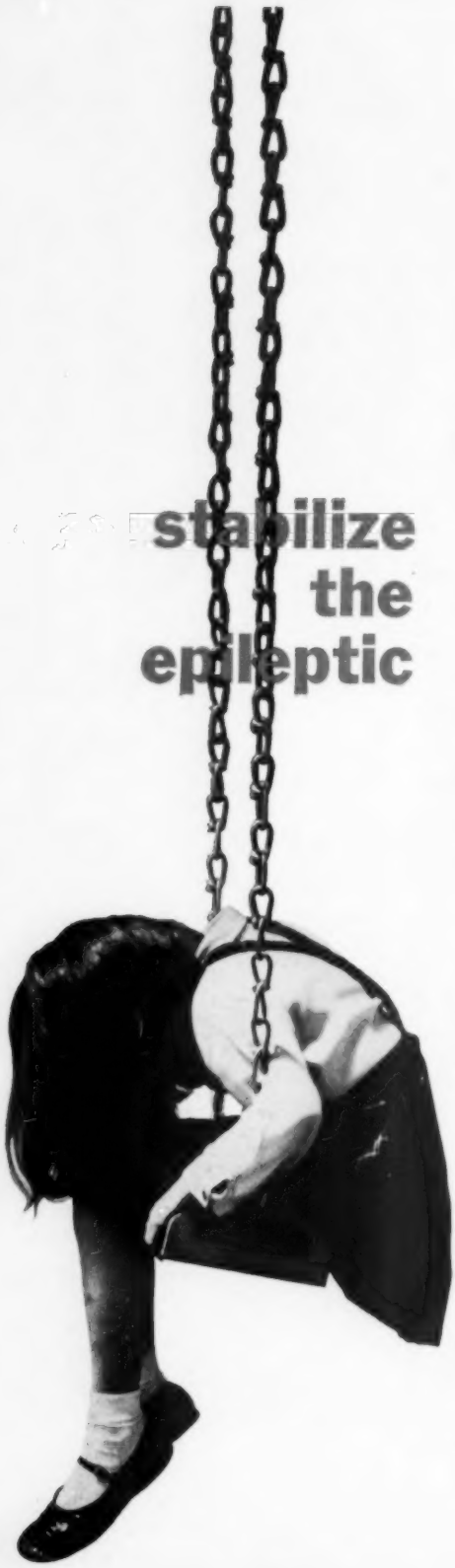
*rich, creamy lather with improved*

# LOWILA® CAKE

Lowila Cake now lathers like soap. Its new luxuriant lather is creamy, more abundant . . . more pleasing to patients. It cleans tender or dermatitic skin with virtually no irritation.

*In bar form. Write for samples.*

WESTWOOD PHARMACEUTICALS
Buffalo 13, NEW YORK



# stabilize the epileptic

**Better control of seizures and better understanding by parents before a child starts school help a great deal to establish a stable, normal outlook in the epileptic.**

*Highly effective:* In most types of seizures, especially major motor seizures, **MEBARAL** is one of the best-tolerated anticonvulsants for children. This antiepileptic does not tend to lower learning capacity.

*Unsurpassed safety:* Mebaral is not likely to cause hyperactivity in young children. Toxic reactions or ill effects from it are rare.

**MEBROIN®,** combining Mebaral and diphenylhydantoin, is synergistic—and side effects are infrequent. Each relatively tasteless Mebroin tablet contains 90 mg. Mebaral and 60 mg. diphenylhydantoin. Mebroin brings maximal control of seizures with minimal toxicity.

*Mebaral dosage:* CHILDREN under 5 years, from ¼ to ½ grain three or four times daily; over 5 years, from ½ to 1 grain three or four times daily. ADULTS, from 6 to 9 grains daily.

*Mebroin dosage:* CHILDREN under 6 years, ½ tablet once or twice daily; over 6 years, 1 tablet two or three times daily. ADULTS, 1 or 2 tablets three times daily (average dosage).

## For epilepsy at any age

# MEBARAL®

*Supplied:* **MEBARAL** tasteless tablets of 200 mg. (3 grains), 100 mg. (1½ grains), 50 mg. (¾ grain), 32 mg. (½ grain). Bottles of 100.

**MEBROIN** virtually tasteless tablets. Bottles of 100.

Winthrop LABORATORIES NEW YORK 18, N. Y.

Mebaral (brand of mephobarbital) and Mebroin, trademarks reg. U. S. Pat. Off.